Original Article

Transient elastography for the prevalence of non-alcoholic fatty liver disease in patients with type 2 diabetes: Evidence from the CORDIAL cohort study

Assim A. Alfadda[1,2], Suphia M. Sherbeeni[3], Adel N. Alqutub[4], Abdullah S. Aldosary[5], Nasser M. Aldaghri[6], Simon D. Taylor-Robinson[7], Saleh A. Alqahtani[8,9], Rukhsana Gul[1], Abdulrahman M. Almaghamsi[10]

[1]Obesity Research Center, [2]Department of Medicine, College of Medicine, King Saud University, Riyadh, [3]Tadaw Medical Complex and Day Surgery Center, Riyadh, Departments of [4]Gastroenterology and Hepatology and [5]Medical Imaging Administration, King Fahad Medical City, Riyadh, [6]Chair for Biomarkers of Chronic Diseases, Biochemistry Department, College of Science, King Saud University, Riyadh, Saudi Arabia, [7]Department of Surgery and Cancer, St. Mary's Hospital Campus, Imperial College London, London, United Kingdom, [8]Liver Transplant Center, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia, [9]Division of Gastroenterology and Hepatology, Johns Hopkins University, Baltimore, MD, USA, [10]Obesity, Endocrine and Metabolism Center, King Fahad Medical City, Riyadh, Saudi Arabia

Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is highly prevalent in patients with type 2 diabetes mellitus (T2DM). This study aimed to investigate the prevalence of NAFLD among Saudi patients with T2DM using transient elastography.

Methods: A total of 490 patients with T2DM who attended diabetes and primary care clinics were recruited. Controlled attenuation parameter (CAP) and liver stiffness measurements (LSM) were obtained via FibroScan to assess steatosis and fibrosis.

Results: Of the examined 490 patients with T2DM, 396 (80.8%) had hepatic steatosis (CAP ≥248 dB/m): 326 (66.5%) had severe steatosis (CAP ≥280 dB/m), while 41 (8.4%) and 29 (5.9%) had mild (CAP ≥248 to <268 dB/m) and moderate steatosis (CAP ≥268 to <280 dB/m), respectively. Of the 396 patients with steatosis, only 35 (8.8%) had LSM ≥7.9 kPa, suggesting the presence of fibrosis, while 361 (91%) had LSM <7.9 kPa, indicating the absence of fibrosis. Increased body mass index (BMI), waist circumference, systolic blood pressure (SBP), and alanine aminotransferase (ALT) were positively associated with both steatosis and fibrosis. After adjusting for age and gender, data from logistic regression analysis demonstrated BMI, waist circumference, SBP, ALT, and high-density lipoprotein (HDL) as significant independent factors for steatosis, while SBP was the only significant predictor associated with fibrosis.

Conclusions: Our results demonstrate an increase in prevalence of NAFLD in Saudi patients with T2DM, based on transient elastography and CAP score. The risk of NAFLD appears to be higher in T2DM patients

Address for correspondence: Prof. Assim A. Alfadda, Obesity Research Center, College of Medicine, King Saud University, P. O. Box 2925 (98), Riyadh - 11461, Saudi Arabia.
E-mail: aalfadda@ksu.edu.sa

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with abdominal obesity, elevated SBP, and increased ALT levels, which supports the screening of these conditions in patients with T2DM.

Keywords: FibroScan, fibrosis, non-alcoholic fatty liver, steatosis, type 2 diabetes

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a clinical condition that has reached epidemic proportions, affecting nearly 25% of individuals globally, with the highest prevalence in the Middle East (32% of the population).[1] It embodies a spectrum of chronic hepatic diseases that occur in the absence of alcohol consumption, and represents a major cause of hepatocellular carcinoma (HCC) and liver transplant worldwide.[2,3] NAFLD is associated with an increased risk of metabolic and cardiovascular disorders, such as abdominal obesity, high blood pressure, dyslipidemia, insulin resistance and type 2 diabetes mellitus (T2DM).[4] NAFLD can be subcategorized as non-alcoholic fatty liver (NAFL), affecting 10 to 22% of patients with NAFLD, and a more severe and progressive form called non-alcoholic steatohepatitis (NASH), which affects 20%–30% of patients with NAFLD.[5,6] NAFL is characterized by simple hepatic steatosis, while NASH is defined as steatosis with lobular inflammation and hepatocyte ballooning, which may progress to fibrosis.[7] NASH may ultimately enhance the risk of end-stage liver disease, such as cirrhosis, and hepatocellular carcinoma (HCC) in 10%–20% of patients, and liver-related mortality, especially in patients with poor metabolic health.[8]

Recently, several factors including a sedentary lifestyle, rapid urbanization, and unhealthy eating patterns, have led to an increase in the prevalence of metabolic risk factors such as obesity and T2DM. Indeed, in Saudi Arabia, the number of people with T2DM has increased immensely over the past three decades.[9] An analysis conducted by Alswat *et al*.[10] to assess the burden of NAFLD in Saudi Arabia estimated a rise in the prevalence of NAFLD to 48% by 2030. Another retrospective analysis revealed a 40% increase in the prevalence of NAFLD in patients referred to the hepatology clinic in Taif region.[11] Studies have shown that NAFLD is prevalent in more than 60% of patients with T2DM globally.[12] T2DM is considered to be one of the major clinical predictors for the progression of advanced fibrosis in patients with NAFLD. It is important to note that the prevalence of NAFLD might further accelerate in future due to a parallel rise in the rates of T2DM and obesity.

The diagnostic tools used to confirm hepatic steatosis in patients with NAFLD are critical for the management of T2DM, because once NAFLD is detected in patients with diabetes, intensive monitoring and adjustment of treatment plans are crucial to avoid a poor prognosis. Liver biopsy remains the gold standard tool to diagnose NAFLD, as it not only allows various stages of fibrosis to be distinguished, but also provides information on other important parameters, such as inflammation, necrosis, and steatosis. However, liver biopsy is an invasive, painful, and potentially life-threatening procedure, with sampling variations that could lead to errors in fibrosis staging. Thus, liver biopsy may be impractical, unless non-invasive modalities fail to provide diagnostic or prognostic data. Among non-invasive imaging techniques, ultrasonography, though easily accessible, is semi-quantitative and can underestimate the presence of steatosis; in addition, the reported accuracy and reliability are inconsistent across studies.[13] In this regard, transient elastography using FibroScan® (Echosens Ltd, Paris, France) with controlled attenuation parameter (CAP) measurement has emerged as the leading accurate and non-invasive screening technique that can not only measure liver steatosis, but can also provide an accurate evaluation of the degree of fibrosis simultaneously. Transient elastography is considered to be a more accurate method for assessing even mild steatosis owing to its higher sensitivity (80%–85% or more).[14,15] The prevalence of steatosis and fibrosis using transient elastography has been assessed by multiple prospective cohort studies.[16–20]

The rise in the prevalence of NAFLD in patients with T2DM has been reported before; however, the associated risk factors responsible for the increased prevalence of NAFLD in Saudi patients with T2DM have not been clearly elucidated. Furthermore, to the best of our knowledge, the prevalence of transient elastography–defined steatosis and fibrosis in patients with diabetes has not yet been reported in Saudi Arabia. Thus, in the current study, we aimed to investigate the prevalence of steatosis and fibrosis using FibroScan®, and assess the associated risk factors in a multicenter cohort of Saudi patients with T2DM.

PATIENTS AND METHODS

Study population

This study included patients from the longitudinal cohort

study of Saudi patients with T2DM. The COhoRt of patients with DIAbetes and non-alcoholic Fatty Liver disease (CORDIAL) study began in 2015, and included Saudi patients aged 18–60 years who were diagnosed with T2DM and followed up regularly in the diabetes clinics or primary care clinics. The aim of this cohort was to determine the prevalence and natural history of hepatic steatosis in patients with T2DM. The cohort recruited patients from King Fahad Medical City (KFMC) and affiliated primary care centers in Riyadh, Saudi Arabia. The cohort was approved by the Institutional Review Board at KFMC (study number: 12-344), and all patients provided written, informed consent prior to recruitment. The study was conducted in accordance with the ethical principles for medical research on human subjects adopted by the 18th World Medical Association General Assembly, and the Declaration of Helsinki, 1964 and its subsequent amendments. Patients were excluded from the cohort if they tested positive for hepatitis B surface antigen or had antibodies against hepatitis C virus, were diagnosed with other chronic liver diseases (e.g., hemochromatosis, primary biliary cholangitis, or autoimmune hepatitis), known to have pre-existing hepatic or extrahepatic malignancy, or were consuming >20 g of alcohol per day. The patients will be prospectively followed up for 10 years for metabolic, renal, hepatic, and cardiovascular complications. This paper reports the results of the baseline hepatic assessment for 490 patients.

Clinical and laboratory data collection

The participant characteristics and anthropometric indices, including age, sex, body weight, height, waist circumference, body mass index (BMI), and blood pressure were obtained. Past medical history and social habits (smoking and alcohol intake) were recorded. BMI was calculated as body weight (kg) divided by body height (m^2). Waist circumference was measured at the midpoint between the iliac crest and the lowest rib with a tape all around the body. Blood pressure was measured using the patients' right arm, in the sitting position, after resting for at least 15 min. Blood was sampled for laboratory assays after the patients had fasted for eight hours overnight. Fasting blood glucose, serum lipids, and liver function were measured using Abbott–Architect Plus, a clinical chemistry autoanalyzer (Abbott, Abbott Park, IL, USA). Glycated hemoglobin (HbA1c) determination was performed using D-100®, a high-performance liquid chromatography analyzer (Bio-Rad Laboratories, Hercules, CA, USA).

Liver FibroScan examination

FibroScan® 502 and FibroScan® 530 Compact, with two probes, Medium (M+) and Extra-large (XL+) (Echosens Ltd, Paris, France), were used for measuring CAP (as surrogate measure of liver fat content) and liver stiffness measurement (LSM, as a surrogate measure of hepatic fibrosis). The device estimates liver steatosis in decibel/meter (dB/m) and liver stiffness in kilopascal (kPa). CAP and LSM were obtained simultaneously in each examination. The type of probe required for each participant was selected by an automatic probe selection tool embedded within the FibroScan® operating software. A successful vibration-controlled transient elastography (VCTE) exam was defined by the acquisition of ten successful measurements, where the interquartile range of the LSM did not exceed 30% of the median LSM. Therefore, an "uninterpretable" VCTE examination encompassed failures on one or both accounts. Each patient underwent VCTE examination after three hours of fasting. All VCTE examinations were performed by two experienced physicians.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS Inc., IBM, Armonk, New York, USA). The results were expressed as frequencies (numbers and percentages) for categorical variables, and as mean, standard deviation, range, and confidence intervals (CIs) for continuous variables. Measurement of the strength and direction of the relationship/correlation between two continuous and categorical variables was performed using the Pearson correlation test and the Chi-squared (χ^2) test, respectively. An independent *t* test was performed to determine the difference between two means. One-way analysis of variance (ANOVA) was used to determine significant differences in the means of the laboratory results according to grades of steatosis and fibrosis. Logistic regression analysis was performed to determine the most significant variables associated with steatosis and fibrosis. The odds ratios and 95% CIs were obtained to signify the strength of the association between variables and steatosis and fibrosis. All *P* values were two-tailed, and statistical significance was set at $P < 0.05$.

RESULTS

Baseline characteristic of the patients

The baseline characteristic of the patients who attended the clinic, with reference ranges used in the laboratory, are shown in Table 1. In total, 495 patients diagnosed with T2DM were included in this cohort study, among whom five cases were excluded as four were diagnosed with hepatitis C virus and one was diagnosed with hepatitis B virus. Therefore, 490 patients with T2DM who fulfilled the inclusion

criteria were included in the final analysis. The mean age of the included patients was 49.9 (range, 18–60) years, and comprised of 262 men (53.5%) and 228 women (46.5%). The mean BMI and waist circumference of the study participants were 32.7 ± 5.7 kg/m^2 and 109.8 ± 11.3 cm, respectively. Three hundred twenty-five participants (69.7%) were obese (BMI ≥30 kg/m^2), and of those, 302 (92.9%) were centrally obese according to their waist circumference measurements. In our cohort, the duration of diabetes ranged from 1 to 53 years with a mean and median of 11.46 years and 10 years, respectively. The mean of fasting blood glucose of the patients was 8.8 ± 3.5 mmol/L, and it was comparable in both the sexes. The patients' glycosylated hemoglobin (HbA1c) mean value was 8.3%±1.9% suggesting poor glycemic control.

Factors associated with steatosis grade using CAP values

Table 2 shows the clinical and biochemical characteristics of the patients, according to steatosis grading. The optimal cutoff values for classifying steatosis grades were as follows: S0 (CAP <248 dB/m) for no steatosis; S1 (CAP 248 to <268 dB/m) for mild steatosis; S2 (CAP 268 to <280 dB/m) for moderate steatosis; and S3 (CAP ≥280 dB/m) for severe steatosis.[21] The mean and median CAP values were 302.1 (55.9) dB/m and 304.5 dB/m (range, 100 to 400 dB/m). According to the CAP scores, the majority of the patients were in the S3 category (326, 66.5%), while the remaining patients were in the S0 (94, 19.2%), S1 (41, 8.4%), and S2 (29, 5.9%) categories. Univariate analysis demonstrated a positive association between increasing CAP values and systolic blood pressure (SBP), BMI, body waist circumference, and serum alanine aminotransferase (ALT) [Table 2]. As the steatosis grade increased, the proportion of participants with obesity also increased and was highest at 70.8% in participants with S3 grade steatosis. Overall, a greater proportion of women had obesity compared to men (179/228, 78.5% vs 146/262, 55.7%; $P < 0.001$). However, there were no significant differences between men and women with respect to steatosis (67.2% and 65.8% had a CAP ≥280, respectively). Furthermore, no association was observed between increasing steatosis grade and waist–hip ratio. A negative association was observed between high-density lipoprotein (HDL) and increasing steatosis grade ($P < 0.001$), but total cholesterol, low-density lipoprotein (LDL), and triglycerides showed no significant association. Multivariate analysis showed significant independent variables for steatosis that included waist circumference (F [1,90] = 1.80, $P = 0.006$), ALT (F [1,77] = 1.06, $P < 0.001$), ALP (F [1,94] = 1.56, $P = 0.029$, and triglycerides (F [1,211] = 1.30, $P = 0.021$). Logistic regression analysis showed elevated BMI, waist circumference, SBP, ALT, and lower HDL as independent predictors for steatosis after adjusting for age and gender [Table 3].

Factors associated with fibrosis using LSM values

Table 4 shows the clinical and biochemical characteristics of patients according to fibrosis grading. The optimal cutoff values for classifying fibrosis grades were as follows: F0–F1, < 7.9; F2, 7.9 to <8.8; F3, 8.8 to <11.7; and F4 ≥11.7 kPa.[1] Of the 396 steatosis patients with valid LSM values, the majority were in the F0–F1

Table 1: Clinical and laboratory characteristics of 490 T2DM patients with reference ranges

Parameters	Reference range	Mean (SD)	Males *n*=262	Females *n*=228	*P*
Age, years	-	49.9 (7.5)	50.2 (7.9)	49.7 (6.9)	0.478
Systolic blood pressure, mmHg	<120	129.6 (16.6)	129.9 (15.9)	129.2 (17.5)	0.626
Diastolic blood pressure, mmHg	<80	75.7 (10.5)	77.9 (10.1)	73.2 (10.4)	<0.001
BMI, kg/m^2	18.5-24.9	32.7 (5.7)	30.9 (5.2)	34.6 (5.8)	<0.001
Waist circumference, cm	≤102 Males ≤88 Females	109.8 (11.3)	106.9 (13.5)	104.6 (12.6)	0.049
Waist/hip ratio	≤0.90 Males ≤0.80 Females	0.96 (0.08)	1.0 (0.05)	0.9 (0.1)	<0.001
ALT, IU/L	0.0-55.0	26.5 (18.5)	31.4 (21.7)	21.0 (11.5)	<0.001
AST, IU/L	5.0-34.0	21.9 (13.7)	22.7 (12.8)	20.9 (14.7)	0.159
GGT, IU/L	12.0-64.0	37.3 (55.8)	40.3 (57.2)	33.5 (53.9)	0.214
Serum bilirubin, μmol/L	3.4-20.5	9.0 (8.8)	10.5 (11.4)	7.2 (3.6)	<0.001
Alkaline phosphatase, IU/L	40.0-150.0	75.5 (27.1)	74.7 (30.4)	76.3 (22.7)	0.519
Serum albumin, g/L	35.0-52.0	41.6 (2.8)	42.2 (2.9)	41.0 (2.5)	<0.001
Fasting Blood Glucose, mmol/L	3.89-5.83	8.8 (3.5)	8.9 (3.3)	8.9 (3.5)	0.973
HbA1c, %	<6.50	8.3 (1.9)	8.2 (1.7)	8.4 (2.1)	0.338
Total cholesterol, mmol/L	≤5.180	4.3 (1.1)	4.1 (1.0)	4.6 (1.2)	<0.001
HDL-cholesterol, mmol/L	>1.55	1.1 (0.3)	1.0 (0.2)	1.2 (0.4)	<0.001
LDL-cholesterol, mmol/L	<2.60	2.7 (0.9)	3.1 (8.4)	2.8 (0.9)	0.559
Triglycerides, mmol/L	≤1.70	1.6 (1.3)	1.6 (0.9)	1.7 (1.6)	0.285

BMI: body mass index, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: γ-glutamyl transpeptidase, HDL: high-density lipoprotein, LDL: low-density lipoprotein

Table 2: Clinical and biochemical variables according to steatosis grade of 490 T2DM patients

Continuous variables	Steatosis grade				*P*
	S0 (<248) *n*=94	S1 (248 to <268) *n*=41	S2 (268 to <280) *n*=29	S3 (≥280) *n*=326	
Age, years	49.6 (8.2)	49.6 (7.5)	49.6 (8.7)	50.1 (7.1)	0.926
Systolic blood pressure, mmHg	125.3 (16.1)	125.7 (16.0)	125.6 (16.0)	131.8 (16.6)	0.001
Diastolic blood pressure, mmHg	74.0 (11.2)	75.9 (11.4)	75.1 (12.8)	76.2 (9.9)	0.402
BMI, kg/m^2	30.5 (6.6)	32.5 (4.1)	32.0 (4.9)	33.4 (5.6)	0.001
Waist circumference, cm	102.2 (12.4)	103.1 (11.2)	103.0 (10.8)	107.3 (12.3)	0.001
Waist/hip ratio	0.95 (0.1)	0.95 (0.08)	0.92 (0.09)	0.97 (0.08)	0.075
ALT, IU/L	23.7 (23.1)	19.7 (7.7)	22.4 (13.4)	28.5 (17.9)	0.005
AST, IU/L	20.7 (14.4)	17.8 (5.0)	20.4 (8.6)	22.9 (14.5)	0.132
GGT, IU/L	43.2 (75.7)	31.5 (37.8)	26.1 (16.3)	37.1 (52.8)	0.547
Serum bilirubin, μmol/L	8.9 (4.4)	9.2 (5.8)	9.5 (6.8)	9.0 (10.2)	0.985
Alkaline phosphatase, IU/L	77.7 (39.9)	72.2 (18.5)	74.6 (16.8)	75.3 (24.0)	0.733
Serum albumin, g/L	41.4 (3.1)	41.2 (2.4)	41.1 (2.6)	41.8 (2.7)	0.265
Fasting blood glucose, mmol/L	9.0 (3.7)	8.0 (3.4)	7.9 (2.7)	9.0 (3.5)	0.174
HbA1c, %	8.1 (2.0)	8.1 (2.1)	8.1 (1.8)	8.4 (1.9)	0.320
Total cholesterol, mmol/L	4.3 (1.2)	4.3 (1.2)	4.4 (1.0)	4.4 (1.1)	0.960
HDL-cholesterol, mmol/L	1.2 (0.5)	1.2 (0.3)	1.2 (0.4)	1.0 (0.3)	<0.001
LDL-cholesterol, mmol/L	2.7 (0.9)	2.7 (1.0)	2.7 (0.9)	2.7 (0.9)	0.884
Triglycerides, mmol/L	1.4 (0.7)	1.4 (0.8)	1.6 (0.8)	1.8 (1.5)	0.057
Elasticity, kPa	5.5 (4.5)	4.6 (2.1)	4.8 (1.7)	5.9 (4.8)	0.176

Values are expressed as mean (standard deviation or SD), Test used: one-way analysis of variation (ANOVA) BMI: body mass index, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: γ-glutamyl transpeptidase, HDL: high-density lipoprotein, LDL: low-density lipoprotein.

category (361, 91%) with a mean LSM (>7.9 kPa), while the remaining patients were in F2 (3, <1%), F3 (16, 4%), or F4 (16, 4. %). Only 35 patients (8.8%) in F2–F4 category had LSM ≥7.9 kPa, suggesting the presence of fibrosis, while 361 patients (91%) had absent or low-grade fibrosis with LSM <7.9 kPa. There was a significant association between fibrosis and BMI ($P < 0.001$), body waist circumference ($P < 0.001$), SBP ($P < 0.001$), ALT ($P = 0.001$), aspartate aminotransferase (AST) ($P = 0.001$), γ-glutamyl transpeptidase (GGT) ($P = 0.018$), and alkaline phosphatase (ALP) ($P < 0.001$). Multivariate analysis showed significant independent variables for fibrosis, which included age (F [1,58] = 1.70, $P = 0.016$), SBP (F [1,83] = 2.63, $P < 0.001$), ALT (F [1,77] = 3.11, $P < 0.001$), ALP (F [1,94] = 3.66, $P < 0.001$), Albumin (F [1,92] = 2.53, $P < 0.001$). Logistic regression analysis showed SBP ($P = 0.001$) as an independent risk factor associated with fibrosis after adjusting for age and gender (OR: 1.038, 95% CI: 1.015–1.062).

DISCUSSION

The increased incidence and prevalence of T2DM is one of the most challenging health concerns in the Kingdom of Saudi Arabia.[22,23] Considering the increased risk of developing NAFLD among patients with T2DM,[24] studying the prevalence of steatosis and fibrosis, which are major causes of chronic liver diseases among this population, is an urgent unmet clinical need. To the best of our knowledge, no previous study from Saudi Arabia has reported the prevalence of transient elastography–defined NAFLD and their associated risk factors in patients with T2DM.[25] In our cohort study of 490 patients with T2DM aged 18–60 years, we demonstrated the prevalence of steatosis by CAP ≥280 dB/m and fibrosis by LSM ≥7.9 kPa using FibroScan. In consensus with previous studies, our data demonstrated that the prevalence of steatosis was high among patients with T2DM.[26,27]

We observed that 80.8% of the patients in our cohort had steatosis (CAP ≥248), and 66.5% displayed S3 grade or severe steatosis (CAP ≥280). Similarly, the prevalence of steatosis in a T2DM cohort screened by transient elastography has been reported from Italy (72%),[16] Turkey (94.3%),[17] Hong Kong (72.8%),[18] and Malaysia (72.4%).[19] Moreover, in a study on patients with T2DM, Sima *et al.* reported steatosis in 93.8% of patients with CAP ≥232.5 dB/m and severe

Table 3: Logistic regression analysis for variables associated with steatosis with adjustments for age and gender

Factors	Unadjusted		Adjusted	
	OR (95% CI)	*P*	OR (95% CI)	*P*
BMI	1.089 (1.262-2.856)	0.002	1.075 (1.039-1.144)	0.012
Systolic blood pressure	1.980 (1.336-2.936)	0.001	1.020 (1.007-1.034)	0.004
Waist circumference	2.785 (1.693-4.583)	<0.001	2.153 (1.072-4.327)	0.031
ALT	5.654 (1.309-24.418)	0.020	1.023 (1.006-1.041)	0.009
HDL	0.431 (0.292-0.635)	<0.001	0.268 (0.118-0.609)	0.002

BMI: body mass index, ALT: alanine aminotransferase, HDL: high-density lipoprotein

steatosis in 70.8% of patients with CAP ≥290 dB/m.[20] The observed variations between studies might have occurred due to differences in our cutoff values for CAP for the diagnosis of NAFLD. Notably, the prevalence of NAFLD in our cohort was higher than previous reports, ranging from 47.8% (95% CI, 41.1%–54.6%) to 72.8% (95% CI, 66.6%–78.1%) in Saudi patients with T2DM. These studies used biochemical data and abdominal ultrasound examination for screening NAFLD. Thus, the actual occurrence of NAFLD in patients with diabetes has not been conclusively determined in Saudi Arabia. This is considered to be due to discrepancies attributed to the poor sensitivity of ultrasonography to detect mild forms of steatosis in comparison to transient elastography.

Many previous studies have suggested that obesity (BMI ≥ 30) is a common risk factor for steatosis.[28] The results of the present study also revealed a reliable association of BMI and waist circumference with steatosis. Similar associations of central and visceral obesity with NAFLD were revealed in previous studies.[28,29] In our cohort, the prevalence of obesity was nearly 69.7%, and 70.2% of obese patients had steatosis with CAP ≥ 280 dB/m. Despite the fact that the prevalence of obesity was lower in males than females (55.7% vs 78.5%, $P < 0.001$), our data displayed no significant differences between the genders in the prevalence of steatosis or fibrosis among obese patients. Both univariate and bivariate analysis demonstrated that BMI and waist circumference were associated with steatosis. Moreover, data from logistic regression analysis also revealed BMI and waist circumference as independent risk factors for predicting steatosis among T2DM individuals. An association of higher BMI with higher CAP was previously reported by a French study that assessed the prevalence of hepatic steatosis in 705 patients with T2DM.[30]

The majority of patients with fatty liver are asymptomatic, and liver abnormalities are usually detected by assessment of serum liver enzymes in routine laboratory tests. Among serum amino transferases, changes in ALT levels are commonly used to screen NAFLD. Our data showed an increased association of ALT with increasing grades of steatosis, and significantly increased levels of ALT in participants with steatosis. However, despite the increased prevalence of NAFLD and higher ALT levels, AST and GGT levels were not associated with steatosis grade, and their levels were not significantly different in patients with or without steatosis. These data suggest that serum liver enzymes tests are insufficient to detect fatty liver in patients with T2DM. Recent studies have suggested that AST levels do not correlate well with the degree of hepatic steatosis, and may even be normal in patients with high grades of steatosis.[31] Indeed, studies have demonstrated that these enzymes may not be elevated in all cases with liver steatosis, and that the level of amino transferases may not reliably predict the extent of NAFLD.[32] Other factors on blood analysis found to be significantly associated with steatosis in the current study were HDL levels, whereas total cholesterol, LDL, and triglycerides were not associated with increasing steatosis. However, bivariate analysis revealed a significant correlation between HDL and triglycerides with

Table 4: Clinical and biochemical variables according to fibrosis grade of 396 T2DM patients

Parameters	Fibrosis stage				ANOVA
	F0-F1 *n*=361	F2 *n*=3	F3 *n*=16	F4 *n*=16	*P*
Age, years	49.9±7.3	44.0±15.1	52.0±5.8	50.9±5.6	0.321
Systolic blood pressure, mmHg	129.8±16.5	134.0±2.8	134.8±13.7	144.6±17.6	0.004[a]
Diastolic blood pressure, mmHg	75.9±10.3	77.0±5.7	75.4±10.3	78.3±10.6	0.831
BMI, kg/m^2	32.7±4.8	39.9±11.5	38.4±9.5	36.1±8.1	<0.001[b]
Waist circumference, cm	105.6±11.3	118.8±10.9	121.8±24.9	115.2±20.9	<0.001[c]
Waist/hip ratio	0.96±0.08	0.99±0.06	0.96±0.08	0.98±0.09	0.655
ALT, IU/L	26.77±16.9	25.0±10.8	28.6±21.9	35.3±17.3	0.273
AST, IU/L	21.8±13.1	20.3±5.9	26.5±21.9	26.1±13.5	0.381
GGT, IU/L	34.8±51.3	20.5±0.7	38.6±19.4	56.4±40.9	0.408
Serum bilirubin, µmol/L	9.1±10.0	8.5±3.9	8.5±3.9	8.5±3.8	0.989
Alkaline phosphatase, IU/L	74.3±23.1	77.0±8.2	82.4±22.9	80.8±24.2	0.392
Serum albumin, g/L	41.8±2.7	41.1±1.1	40.4±2.8	40.9±2.0	0.145
Fasting blood glucose, mmol/L	8.7±3.2	8.5±3.4	10.9±4.6	8.9±3.9	0.083
HbA1c, %	8.3±1.9	7.6±0.9	9.1±2.3	8.4±1.8	0.425
Total cholesterol, mmol/L	4.3±1.1	5.4±1.4	4.5±0.8	4.3±1.1	0.372
HDL-cholesterol, mmol/L	1.1±0.3	0.8±0.1	1.1±0.2	1.0±0.2	0.299
LDL-cholesterol, mmol/L	3.1±7.1	3.8±1.5	2.8±0.6	2.6±0.9	0.989
Triglycerides, mmol/L	1.7±1.5	2.5±1.3	1.7±0.9	1.7±0.8	0.824
CAP, dB/m	318.5±40.4	342.0±30.5	352.6±36.6	346.9±44.2	0.001[d]

Post hoc analysis: a - Significant difference between F0F1 and F4 (P=0.003); b - Significant difference between F0F1 and F3 (P<0.001); c - Significant differences between F0F1 and F3 (P<0.001) and F0F1 and F4 (P=0.026); d - Significant difference between F0F1 and F3 (P=0.06) and between F0F1 and F4 (P=0.049). BMI: body mass index, ALT: alanine aminotransferase, AST: Aspartate aminotransferase, GGT: γ-glutamyl transpeptidase, HDL: high-density lipoprotein, LDL: low-density lipoprotein, CAP: Controlled attenuation parameter.

CAP values. In contrast to earlier findings that reported an association between NAFLD and dyslipidemia, our data did not show any significant difference in dyslipidemia between participants with or without steatosis, except for low HDL levels in patients with increasing steatosis, which was shown previously.

The severity of liver fibrosis is a strong predictor for the presence of NAFLD in patients with T2DM. Transient elastography revealed that 91% of our study patients (396) had no fibrosis (F0–F1) with a mean LSM (>7.9 kPa), and 8.8% (35) had LSM ≥7.9, which was suggestive for fibrosis. Among the 35 patients, 3 (<1%) had F2 stage fibrosis, 16 (4%) had F3 stage fibrosis (LSM >8.8), and 16 (4%) had F4 stage fibrosis (LSM ≥11.7). Advanced stage fibrosis (F3 and F4) was detected in 32 patients (8%) which is comparable to the figures in previous studies that reported the prevalence of undiagnosed liver fibrosis in patients with T2DM.[33] Based on our data from univariate and bivariate analysis, we conclude that, in our cohort, patients with fibrosis (LSM ≥7.9 kPa) predominantly comprise obese patients with diabetes, increased BMI, waist circumference, SBP, and elevated liver enzymes such as ALT, AST, GGT, ALP and serum albumin. Moreover, logistic regression analysis after adjusting for age and gender showed a significant independent association between fibrosis and increased SBP.

Our study has some limitations that should be mentioned. First, although FibroScan® is a highly validated tool to assess even mild steatosis with much higher sensitivity, it can also yield unreliable results due to its poor ability to distinguish patients with early stage F0 and F1 fibrosis. Second, liver biopsies, considered to be the gold standard to diagnose NAFLD, were not performed to associate the fibrosis data obtained by transient elastography with histological evaluations. However, liver biopsies are invasive in nature and the data obtained by histological analysis may vary between different clinicians. In contrast, FibroScan® is non-invasive and is able to accurately identify and differentiate between advanced fibrosis stages. Strengths of the study include its usefulness to evaluate the prevalence of transient elastography–defined steatosis and fibrosis in a T2DM population in Saudi Arabia, the comprehensive clinical and biochemical data obtained, and the large number of participants. This represents the first report from the CORDIAL study that attempts to decipher the natural history of NAFLD among patients with T2DM.

In conclusion, the results of our study indicate an increased association of NAFLD with T2DM in Saudi patients using transient elastography. Patients with high BMI and increased SBP are at greater risk of developing NAFLD. Considering growing obesity and increasing prevalence of T2DM, NAFLD is anticipated to increase dramatically and become a serious health concern worldwide. Our data could be applicable to the other populations with comparable prevalence rates for obesity and T2DM. Thus, to alleviate the growing burden, we recommend that efficient preventive strategies be developed to screen all patients with T2DM for NAFLD, in particular patients with associated comorbidities of abdominal obesity, elevated SBP and raised ALT levels. The screening can be done by using non-invasive tests as a routine component of diabetic care.

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Conflicts of interest

There are no conflicts of interest.

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