ORIGINAL ARTICLE

Predictors of Non-Alcoholic Fatty Liver Disease in Type 2 Diabetes

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Non-alcoholic fatty liver disease (NAFLD) is common in patients with type 2 diabetes and its diagnosis in clinics is based on ultrasonography. The aim of this study is to examine the role of some clinical and laboratory variables in predicting NAFLD diagnosed by ultrasonography in patients with Type 2 diabetes.

Material and Methods: The study was performed on 76 consecutive alcohol-negative and hepatitis B and C virus-negative patients, attending an endocrine clinic; all of them had undergone a complete clinical and biochemical work up, including demographic and anthropometric factors, lipid profiles, fasting plasma glucose, glycosilated hemoglobin (HbA1c), liver transaminases and alkaline phosphatase. Using ultrasonography, patients were divided into two groups, one with and the other without NAFLD. A logistic regression model was developed in stepwise manner to evaluate predictors of NAFLD.

Results: Average age was 60±9 years. Forty-nine (64.5%) patients were female. Sixty-three patients (82.9%) had ultrasonography-diagnosed NAFLD. Average body mass index (BMI) was higher in NAFLD patients (29.4±4.4 kg/m2 vs. 24.8±3.8 kg/m2, P<0.05). Among age, gender, FPG, duration of diabetes, triglycerides, waist circumference and BMI, the only independent factor associated with ultrasound-diagnosed NAFLD was BMI [adjusted odds ratio for 25≤BMI<30 kg/m2: 7.8 (95% CI: 1.9 to 32.4); BMI ≥30 kg/m2: 24 (95% CI: 2.6 to 223), P<0.001].

Conclusion: The findings demonstrate that BMI per se can be considered as an independent predictor of NAFLD in patients with type 2 diabetes.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of diseases of the liver ranging from steatosis (i.e. fatty infiltration of the liver) to NASH (i.e. steatosis with inflammation and hepatocyte necrosis to cirrhosis).[1] NAFLD is the most common cause of elevated liver enzymes among adults in the United States[2] and the most common cause of cryptogenic cirrhosis.[3] The prevalence of NAFLD in Western countries is high and there is a trend toward a further increase, with millions of people at risk of advanced liver disease. NAFLD affects approximately 15–30% of the general population, and its prevalence increases steadily to 70–90% in people with obesity or type 2 diabetes.[4-7]

The pathogenesis of NAFLD is multifactorial and it has been suggested that the presence of insulin resistance (IR) is essential for the accumulation of hepatocellular fat.[8] NAFLD has been suggested to be associated with the metabolic syndrome and has been described as the hepatic component of this syndrome.[5,7] The most common risk factors

for the development of steatosis are obesity,[9] diabetes, and hypertriglyceridemia; other causes include toxins, medications, and inborn errors of metabolism.[10]

Given the strong associations between NAFLD and the metabolic syndrome risk factors, patients with NAFLD would be expected to have an increased risk of CVD; however, it has been shown recently that NAFLD is associated with greater overall mortality and predicts the risk of future cardiovascular disease (CVD) events independently of classic and well-known metabolic risk factors.[11] Targher et al., recently,[12] found that type 2 diabetic individuals with NAFLD have significantly higher rates of prevalent coronary, cerebrovascular and peripheral vascular diseases than their counterparts without NAFLD. Moreover, Younossi et al. reported a risk ratio of 3.3 and 22.8 for overall mortality and mortality related to liver diseases, respectively, in patients with diabetes and NAFLD in comparison to non-diabetic subjects with NAFLD.[13]

In view of the increased risk of CVD events and mortality and availability of various treatment modalities, the timely diagnosis of NAFLD in type 2 diabetes is of critical importance. Biopsy is the only definitive method of diagnosing NAFLD and to some extent, determining its prognosis;[14] however, the value of liver biopsy for diagnosis of NASH as a subgroup of NAFLD in routine clinical practice is controversial.[14] It has been reported that ultrasonography can accurately identify steatosis with a sensitivity of 94% and a specificity of 84%,[15] and has a sensitivity and specificity of 77% and 89%, respectively, in detecting increased fibrosis.[16] Moreover, high level of agreement between ultrasonography and computed tomography has been reported.[17]

Considering the high prevalence of NAFLD in type 2 diabetics, prediction of potential NAFLD patients in clinical settings is valuable. With regard to the acceptable diagnostic accuracy of ultrasound scanning,[18] we aimed at addressing the best predictors of ultrasonography-diagnosed NAFLD in patients with type 2 diabetes. We also aimed at examining the association between ultrasonography-diagnosed NAFLD and the metabolic syndrome in these patients.

Materials and Methods

Patients and Setting: Seventy-six outpatients aged ≥ 40 years with known type 2 diabetes, using oral glucose-lowering agents, and referred for the first time to the endocrine clinic of the Imam Hussein General Hospital, Shaheed Beheshti University of Medical Sciences (SBUMS) (Tehran, Iran), were enrolled consecutively in this cross-sectional study from November 2005 to March 2006. All patients provided written informed consent before participation in this study, which was approved by institutional ethics committees (Research Institute for Endocrine Sciences, SBUMS) and was conducted in accordance with the principles of the Declaration of Helsinki.

We excluded patients with histories of liver diseases such as cirrhosis, alcohol consumption of over 20 gr per day for women and over 30 gr per day for men, or severe or debilitating diseases such as cancers and severe anemia (hemoglobin<10 mg/dL); also excluded were patients with clinical and subclinical hypothyroidism, patients with blood sample positive for HCV antibody or positive for HBS antigen, patients with a serum iron to total iron binding capacity ratio of over 50 and patients with any history of taking tamoxifen, corticosteroids, amiodarone, oral contraceptives, valproic acid, diltiazem, nifedipine, methotrexate, and tetracycline.

Patient evaluation: Data collection, including medical history, demographic, clinical and paraclinic information, was carried out according to fixed protocols, using structured closed question datasheets. Weight was measured with light clothing and without shoes using a Seca 707 weighing machine (range: 0.1-150 kg) with an accuracy of up to 100 gr. Height was measured without shoes using a tape stadiometer with a minimum

measurement of 1 mm. Body mass index (BMI) was calculated by dividing weight (in kilograms) by height squared (in meters). Standing waist circumference was measured at the level of the umbilicus with a flexible tape; waist circumference ≤102 cm in men and ≤88 cm in women considered as normal.

Blood pressure was measured twice after participants were seated for 15 min using a standard mercury sphygmomanometer. There was at least a 30-second interval between these two separate measurements, the mean of which was considered as the blood pressure. The systolic blood pressure (SBP) was defined as the appearance of the first sound (Korotkoff phase 1) and the diastolic blood pressure (DBP) was defined as the disappearance of the sound (Korotkoff phase 5) during deflating the cuff.

Laboratory Measures: Blood samples, drawn between 7:00 and 9:00 AM into vacutainer tubes from all study participants after 12-14 hours of overnight fasting, were centrifuged within 30-45 minutes of collection, and were assessed for total serum cholesterol (TC), high density lipoprotein (HDL), triglycerides (TG), fasting plasma glucose (FPG), glycosilated hemoglobin (HbA1c), complete blood count, liver transaminases, alkaline phosphatase, thyroid function tests, serum iron level, total iron binding capacity (TIBC) and markers for hepatitis B and C viruses infection.

All blood lipid analyses were carried out at the laboratory of the Research Institute for Endocrine Sciences on the day of blood collection using TC and TG kits (TC: CV interassay=2%, TG: CV interassay=1.6%, Pars Azmoon Co., Iran). TC and TG were assayed using enzymatic colorimetric tests with cholesterol esterase and cholesterol oxidase, and glycerol phosphate oxidase, respectively. HDL-C was measured after precipitation of the apolipoprotein B100 containing lipoproteins with phosphotungistic acid and LDL-C calculated with Friedewald formula. FPG was measured using an enzymatic colorimetric method glucose oxidase technique (Pars Azmoon kit, CV interassay =3%). Serum aminotransferases including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by the IFCC method (Pars Azmoon kit, ALT CV interassay=3.8%, AST CV Inter assay=2.8%). Plasma alkaline phosphatase was measured using the DGKC method (Pars Azmoon kit, CV Inter assay=27%). Serum iron level was measured using the SERENE-S method (Biochemistry kit, CV interassay <2.4%) and total iron binding capacity (TIBC) was measured after precipitation of magnesium carbonate (Biochemistry kit, CV interassay <2.4%). Hepatitis B and C tests were checked using the ELISA technique (Radim kit). Thyroid function tests including T3 and T4 were done using the radioimmunoassay (RIA) method (Immunotech kit, CV interassay <8.6% for both T3, T4) and TSH was measured by IRMA (Spectria kit, CV interassay <4.4%). HbA1c was measured using the Chromatographic expect photometry method (Biosystem kit, CV interassay < 6.2%).

The interval between the time of diabetes diagnosis and the date of our study was considered as "duration of diabetes". The metabolic syndrome was diagnosed according to the criteria of the Adult Treatment Panel (ATP) III Expert Panel of the US National Cholesterol Education Program (NCEP).[19]

To diagnos the NAFLD and assess the grade of steatosis, liver ultrasound scanning (Hitachi EUB 405 apparatus equipped with a convex 3.5 MHz probe) was performed for all patients within a week after blood sampling by a single experienced sonographist, who was blind to all biochemical characteristics of the participants. Liver steatosis was assessed semi-quantitatively, on a scale of 0-3; 0, absent; 1, mild; 2, moderate; 3, severe. Steatosis was graded on the basis of abnor-

mally intense, high level echoes arising from the hepatic parenchyma, liver kidney difference in echo amplitude, echo penetration into the deep portion of the liver, and clarity of liver blood vessel structure.[20,21]

To convert the values for glucose to mmol/L, the glucose values in mg/dl should be multiplied by 0.05551. Furthermore, to convert the values for triglycerides and cholesterol to mmol/L, their values in mg/dl should be multiplied by 0.01129 and 0.02586, respectively.

Statistical Analysis: Data are expressed as the mean±SD for continuous variables or percentages for categorized variables. Continuous and dichotomized variables were compared using the student t test and the chi-square test, respectively. The independence of the association of variables with the presence NAFLD was assessed by multiple logistic regression analysis in backward conditional method and expressed as odds ratios (OR). For this analysis, age (year), duration of diabetes (year), fasting plasma glucose (mg/dl), waist circumference (reference: normal), plasma triglyceride level (reference: <150 mg/dl) and body mass index (kg/m2) (reference: <25 kg/m2) were used as independent variables and presence (mild to severe) or absence of NAFLD was used as dependent variable.

Likelihood ratios (LR) and their confidence intervals (CI) were calculated to estimate the odds of having NAFLD. LRs express how many times more likely a test result (in this study, for having NAFLD) is to be found in subjects with predicting characteristics; for example, obese subjects compared with normal BMI or triglycerides above the certain cutoff, compared with those with triglycerides below that cutoff. By definition, LR(+) = sensitivity/(1-specificity) and LR(-) = (1-sensitivity)/specificity. All statistical analyses were performed by SPSS software (version 13.0; SPSS Inc. Chicago, Ill, USA). All statistical tests were two sided and differences with probability values <0.05 were considered statistically significant.

Results

The average age was 60±9 years, ranging between 41 and 83 years. Twenty-seven patients were male (35.5%) and 49 patients were female (64.5%). Mean duration of diabetes was 9.8±5.8 years. The HbA1c level was 9.1±1.7 %. In general, 16 patients (21%) had body mass index (BMI)<25 kg/m2; 35 patients (46.1%) had BMI between 25 and 30 kg/m2 and 25 patients (32.9%) had BMI≥30 kg/m2. Furthermore, the means of ALT and AST were 24.8±16.5 IU/L and 23.6±16.4 IU/L, respectively.

Overall, 63 out of 76 patients (82.9%) had NAFLD. Of these, 28 patients (44.4%) had mild steatosis, 27 patients (43%) had moderate steatosis and 8 patients (12.6%) had severe steatosis. In the patients with NAFLD (n=63), average age was 58.7±8.5 years, 22 patients (34.9%) were male, and 41 patients (65.1%) were female.

Table 1 compares the demographical, clinical and laboratory characteristics of patients with NAFLD and those without NAFLD. The average BMI in patients with NAFLD was 29.4±4.4 kg/m^2 compared to 24.8±3.8 kg/m^2 in patients without NAFLD (P<0.05). Furthermore, the average of plasma triglyceride level in patients with NAFLD was significantly higher than that of patients without NAFLD (202±108 mg/dl vs. 126±54 mg/dL; P<0.001) (Table 1). Although the differences were not statistically significant, the mean ALT and AST showed increasing patterns across grades of steatosis from zero to 3 (Table 2).

Table 1. Characteristics of type 2 diabetic patients, with and without non-alcoholic fatty liver disease in ultrasonography

Variable	Non-alcoholic fatty liver disease		P
	Negative (n=13)	Positive (n=63)	
Gender			
Female (%)	8 (38.5)	41 (65.1)	N.S
Male (%)	5 (61.5)	22 (34.9)	
Age (years)	64±9	59±8	<0.05
Duration of diabetes (years)	11.4±5.9	9.4±5.8	N.S
Weight (kg)	64±10.3	74±13.4	<0.05
Height (cm)	160±9.7	156±14.7	N.S
Waist circumference (cm)			
Women	101±18	105±11	N.S
Men	83±14	98±6	N.S
Total	94±19	103±10	N.S
Body mass index (Kg/m2)	24.8±3.8	29.4±4.4	<0.001
ALT (IU/L)	19±10	26±29	N.S
AST (IU/L)	20±4	24±18	N.S
Alkaline Phosphatase (IU/ml)	218±56	216±126	N.S
Fasting plasma glucose (mg/dl)	169±72	170±68	N.S
HbA1C (%)	9.2±1.3	9.1±1.8	N.S
Triglycerides (mg/dl)	126±54	202±108	<0.001
Total cholesterol (mg/dl)	192±30	212±57	N.S
HDL-C (mg/dl)	44±9	39±10	N.S
LDL-C (mg/dl)	128±25	140±50	N.S
Systolic blood pressure (mmHg)	137±26	136±22	N.S
Diastolic blood pressure (mmHg)	80±9	83±14	N.S

To convert the values for glucose to mmol/L, multiply by 0.05551. To convert the values for triglycerides to mmol/L, multiply by 0.01129. To convert the values for cholesterol to mmol/L, multiply by 0.02586. ALT: Alanine aminotransferase; AST: aspartate aminotransferase; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; N.S: Not significant

Table 2. Mean and standard deviation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) according to severity of ultrasonography-diagnosed non-alcoholic fatty liver disease in type 2 diabetes patients

Grade of steatosis	n	ALT (IU/L)	AST (IU/L)
0	13	18.5±9.6	20.1±3.9
1	28	19.3±10.4	21.0±9.1
2	27	29.1±15.7	24.1±19.1
3	8	38.5±18.5	36.1±31.0

On the whole, the metabolic syndrome was seen in 68 patients (89.5%), of whom, 59 patients (86.8%) had NAFLD, whereas, only 43% of patients without the metabolic syndrome had NAFLD (P<0.05). The univariate odds ratio of the metabolic syndrome was 6.56 (95% CI: 1.39 to 30.99) for the presence of ultrasonography-diagnosed NAFLD. The probability of NAFLD increased by addition of components of the metabolic syndrome, since all patients who fulfilled all the five cri-

teria of metabolic syndrome had ultrasonography-diagnosed NAFLD (Chi square linear-by-linear association=8.49, P<0.01) (Fig. 1). Furthermore, the mean grade of steatosis showed an increasing pattern with the addition of the components of metabolic syndrome; however, the differences did not reach to the statistical significance (Table 3).

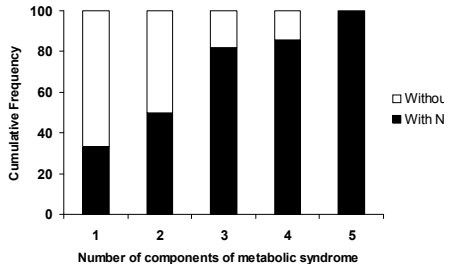

Fig.1. Frequency of ultrasonography-diagnosed non-alcoholic fatty liver disease (NAFLD) according to the number of components of metabolic syndrome in type 2 diabetic patients

Table 3. Mean grade of steatosis and components of the metabolic syndrome in 76 subjects with Type 2 diabetes

Number of components	n	Grade of steatosis
1	3	0.33 ± 0.58
2	5	0.80 ± 0.88
3	23	1.26 ± 0.81
4	35	1.57 ± 0.95
5	10	1.70 ± 0.68

In the multivariate logistic regression model only BMI was an independent predictor of ultrasonography-diagnosed NAFLD; the odds of having NAFLD was 7.8 (95% CI: 1.9-32.4) for 25≤BMI<30 kg/m2, and was 24 (95% CI: 2.6-223) for BMI≥30 kg/m2. Plasma TG level was the next predicting factor after BMI (OR=2.2, 95% CI: 0.4-12.4, P=0.38) (Table 4).

Table 4. Multivariate logistic regression for Non-alcoholic fatty liver disease (NAFLD) in 76 subjects with type 2 diabetes

Variable	NAFLD (n=63)	Total (n=76)	Adjusted odds ratio (95% CI)	P
Fasting plasma glucose (mg/dl)	170±68	170±68	1.0 (0.99 to 1.02)	0.52
Age (years)	58.7±8.5	59.7±8.8	0.93 (0.85 to 1.02)	0.11
Duration of diabetes (years)	9.4±5.8	9.8±5.9	0.98 (0.86 to 1.12)	0.83
Gender				
Male	22 (34.9)	27 (35.5)	1.0	0.41
Female	41 (65.1)	49 (64.5)	0.29 (0.2 to 5.4)	
Triglycerides (mg/dl)				
<150	26 (41.3)	35 (46.1)	1.0	0.38
≥150	37 (58.7)	41 (53.9)	2.2 (0.4 to 12.4)	
Waist circumference [n (%)]				
Normal	19 (30.2)	25 (32.9)	1.0	0.8
Abnormal	44 (69.8)	51 (67.1)	0.7 (0.04 to 13.6)	
Body Mass Index (kg/m2)				
<25	8 (12.7)	16 (21.1)	1.0	
25-<30	31 (49.2)	35 (46.1)	7.8 (1.9 to 32.4)	0.005
≥30	24 (38.1)	25 (32.9)	24 (2.6 to 223)	0.005

Values are numbers (percentages) and continuous data are presented as mean±SD; CI: confidence interval. To convert the values for glucose to mmol/L, multiply by 0.05551. To convert the values for triglycerides to mmol/L, multiply by 0.01129. To convert the values for cholesterol to mmol/L, multiply by 0.02586.

The highest positive LR for having NAFLD was found for BMI≥30 kg/m^2 [LR(+)=4.95; 95% confidence interval (CI): 0.73 to 33.4] and for 25≤BMI<30 kg/m^2 [LR(+)=1.6; 95% CI: 0.68 to 3.8] followed by triglycerides≥150 mg/dl [LR(+)=1.9; 95% CI: 0.82 to 4.4], whilst it was 1.3 (95% CI: 0.37 to 0.95) for abnormal waist circumfer-

ence. The negative LR for the BMI<25 kg/m^2 was 0.17 (95% CI: 0.08 to 0.37).

Discussion

The current study was designed to evaluate the predictors of non-alcoholic fatty liver disease in subjects with type 2 diabetes. The results of this cross-sectional study show that, in tertiary care clinics, approximately 90% of subjects with type 2 diabetes have ultrasound-diagnosed NAFLD and that BMI is the best predicting factor of the NAFLD in such cases. The odds for having NAFLD according to ultrasonography in overweight and obese subjects was 7.8 times and 24 times greater than those with BMI<25 kg/m2, respectively. In addition, ultrasound-diagnosed NAFLD is strongly associated with the presence of metabolic syndrome.

In our study, the crude prevalence of ultrasound-diagnosed NAFLD was 89% in type 2 diabetic subjects, which is in accordance with other reports.[4-7] This prevalence was 62% in Japanese subjects with newly diagnosed diabetes in the study of Jimba et al.,[22] a difference which may be due to the duration of diabetes. Furthermore, although there was no significant difference in HbA1c between our study subjects with and without NAFLD, their high HbA1c, which indicates poor-controlled metabolic status, may be another reason for the difference observed between our study and Jimba's report.[22]

NAFLD has been suggested as a hepatic manifestation of the metabolic syndrome.[23] The prevalence of NAFLD in our type 2 diabetic patients who met the criteria for the metabolic syndrome was 86.7%. In other words, type 2 diabetic patients with metabolic syndrome have a 6.5 times higher risk for having ultrasonography-diagnosed NAFLD. This finding is in accordance with results of a prospective study in 4401 apparently healthy Japanese adults,[24] followed for about 414 days, in which subjects who met the criteria for the metabolic syndrome at baseline had a 4 to 11 times higher risk for future ultrasonography-diagnosed NAFLD. In our study subjects, moreover, the probability of having ultrasonography-diagnosed NAFLD increased with the addition of components of the metabolic syndrome (Fig. 1). Marceau et al.[25] also, reported that with each addition of any of the 5 components of the metabolic syndrome in 551 severely obese patients, the risk of steatosis increased exponentially from 1- to 99-fold.

According to the present study, the best predictors of ultrasonography-diagnosed NAFLD in our type 2 diabetic patients were overall obesity (BMI ≥30 kg/m2) and overweight (25≤ BMI< 30 kg/m2), with adjusted odds ratios of 24 and 7.8, respectively. A large number of studies have suggested obesity as an independent predictor of NAFLD in various settings. Lee et al.[9] studied fifty patients with NAFLD and 100 age and gender matched controls to determine the clinical and metabolic characteristics of ultrasonography-diagnosed NAFLD. A multivariate logistic regression analysis showed that obesity is the only independent factor associated with NAFLD. In a study of 81 Brazilian outpatients with diagnosis of NAFLD by Rocha et al.[26] 42% had increased waist circumference (>102cm in men and >88cm in women) while 93% were overweight or obese. Furthermore, in a longitudinal study of 103 patients with sequential liver biopsy, Adams et al.[27] found that diabetic patients with elevated BMI are at greater risk of fibrosis progression. They also concluded that a decrease in aminotransferases does not seem to indicate an improvement in fibrosis, and can thus provide false reassurance regarding the prognosis.

Considering the point that NAFLD has been suggested as the hepatic manifestation of the metabolic syndrome[23] and is related to insulin resistance, it is surprising that general obesity (measured by BMI) rather than central obesity (measured by waist circumference) was the best predictor of ultrasonography-diagnosed NAFLD. We present here three possible explanations for this finding. First, all of our study subjects had type 2 dia-

betes, indicating that all had some advanced degrees of insulin resistance. Accordingly, this may attenuate the role of central obesity in the multivariate logistic analysis, as the insulin resistance is highly correlated with central fat accumulation. The second explanation for why general obesity and not central obesity was the best predictor of NAFLD is that the cutoff points used for defining the abnormal waist circumference (>102 in men and >88 in women) are not sensitive enough to detect NAFLD; lower cutoffs may be more useful for defining central obesity. Finally, we pooled all the grades of the steatosis into a dichotomous variable, as negative or positive steatosis. This may attenuate the difference between abnormal waist circumference between subjects with moderate and severe steatosis and subjects without steatosis.

Our study has some limitations. One limitation is that, similar to other reports,[9,17] the diagnosis of NAFLD in our study was based on ultrasonography and the exclusion of known causes of chronic liver disease, not confirmed by liver biopsy. However, ultrasonography is the commonest way of diagnosing NAFLD in clinical practice and has acceptable sensitivity and specificity in detecting moderate and severe steatosis in patients with biopsy-proven disease [2,5,16] but not in mild steatosis, in which fat seen on liver biopsy is less than 33%.[28] Another limitation of our method is the study population. All of our study subjects were poorly controlled type 2 diabetic patients, referred for the first time to a tertiary-care clinic. Undoubtedly, study of broader spectrum of type 2 diabetic patients is necessary to increase the external validity of our findings. A third limitation of our method is its cross-sectional design, which does not answer the question of which event is primary, that is, whether NAFLD precedes the insulin resistance and diabetes, or vice versa. Finally, we did not assess insulin resistance, a parameter that is highly correlated to NAFLD.

In conclusion, our results suggest that BMI is the best predictor of ultrasonography-diagnosed NAFLD in type 2 diabetic subjects; therefore, clinicians should be vigilant regarding NAFLD when faced with an obese or an overweight type 2 diabetic patient in routine daily visits, even one with normal liver function tests.

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