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9

**Prevalence of nonalcoholic fatty liver disease and its association with age in patients with type 2 diabetes mellitus**

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10

**Abstract**

**BACKGROUND**

1

Type 2 diabetes mellitus (T2DM) is a risk factor for nonalcoholic fatty liver disease (NAFLD).

**AIM**

To determine the prevalence and clinical correlates of NAFLD in a large cohort of patients with T2DM.

**METHODS**

Four hundred thirty-seven participants with T2DM who consulted at Meijo Hospital from April 2019 to September 2020 and underwent computed tomography (CT) were assessed. The mean age was  $74 \pm 13$  years, and 269 were men. Hepatic attenuation minus splenic attenuation ( $CT_{L-S}$ ) less than 1 Hounsfield unit was considered fatty liver. NAFLD was defined as fatty liver in the absence of significant alcohol consumption and hepatitis virus infection. A multiple logistic regression was used to assess the independent factors associated with NAFLD.

## RESULTS

NAFLD was identified in 25.2% of the participants. Young age (odds ratio [OR] = -0.945; 95% confidence interval [CI]: 0.922–0.969), higher hemoglobin levels (OR = 1.501, 95%CI: 1.278–1.764), lower high-density lipoprotein (HDL) cholesterol levels (OR = 0.971, 95%CI: 0.953–0.989), and the absence of dialysis (OR = 0.109, 95%CI: 0.014–0.856) were independent predictors of NAFLD.

## CONCLUSION

NAFLD was detected with CT in 25.2% of the participants. NAFLD was associated with younger age, higher hemoglobin levels, lower HDL cholesterol levels, and an absence of dialysis.

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) frequently coexists with type 2 diabetes mellitus (T2DM). Both synergistically increase adverse outcomes [1, 2]. NAFLD, T2DM, and obesity are epidemiologically correlated, but their causal interrelationships remain incompletely understood. Liu *et al* proposed the hypothesis of disease subphenotyping in which genetically-driven NAFLD promotes T2DM and central obesity but protects against overall obesity. In contrast, genetically-driven T2DM and obesity increase the NAFLD risk [3].

A meta-analysis showed that the prevalence of NAFLD in patients with T2DM was 56% with ultrasonography (US) and proton magnetic resonance spectroscopy (MRI) [4]. The prevalence of NAFLD in patients with T2DM was highest in Europe (68%), but varies widely depending on the population. Three studies from Japan using US reported that the prevalence of NAFLD was 31%, 69%, and 61% [5-7]. The sensitivity of diagnosing NAFLD varies with the method. The prevalence of NAFLD in patients with T2DM

detected by computed tomography (CT) was 10% in the United States [8], 22% in Turkey [9], and 27% in Japan [10].

In patients with T2DM, NAFLD is associated with an increased risk of overall death [11] but not with liver-related deaths [8]. Meanwhile, in NAFLD patients, T2DM is associated with advanced liver fibrosis [12] and increased mortality related to liver-related deaths [13]. NAFLD in patients with T2DM is associated with an increased risk of cardiovascular disease [7, 9, 10, 14]. However, some reports have denied this association [8, 15-17]. A positive association between NAFLD and nephropathy in patients with T2DM has been reported in some studies [18, 19], while others did not find an association [20-22]. The association of NAFLD with cardiovascular risk and chronic kidney disease in the general population was reported to start in childhood [23, 24].

We studied the prevalence of NAFLD in patients with T2DM in our hospital using CT and determined the factors associated with NAFLD in patients with T2DM.

## **MATERIALS AND METHODS**

### **Study population**

Data of patients with T2DM were retrieved from the hospital database. There were 724 Japanese diabetic patients who consulted at the Federation of National Public Service Personnel Mutual Aid Associations Meijo Hospital from April 2019 to September 2020. We excluded participants who had chronic hepatitis B ( $n = 6$ ) and C ( $n = 28$ ) infections, no assessment of hepatitis B surface antigen and hepatitis C virus antibody ( $n = 105$ ), and an alcohol intake  $\geq 20$  g/day ( $n = 33$ ). We also excluded 115 participants who did not have an abdominal CT examination; thus, 437 participants (269 men and 168 women) were included in the analysis (Figure 1). The mean age was  $74 \pm 13$  years. There were 322 patients treated with oral hypoglycemic agents, 32 with insulin, one with a glucagon-like peptide-1 receptor agonist, and 82 with diet and exercise. All patients had more than a year with T2DM.

The study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of Federation of National Public Service Personnel Mutual Aid

Associations Meijo Hospital (Approval No. 166). Written informed consent was waived because the data were analyzed anonymously based on information stored in the hospital database.

### **Abdominal CT examinations**

A non-enhanced CT was performed using either a 16-section multidetector scanner (Aquilion 16; Canon Medical Systems, Tochigi, Japan), a 64-section multidetector scanner (Aquilion 64; Canon Medical Systems, Tochigi, Japan), or an 80-section multidetector scanner (Aquilion Prime SP/iEdition; Canon Medical Systems, Tochigi, Japan). The CT indications were to screen for diseases in the chest and abdomen in 430 patients. The CT was performed to investigate liver diseases in seven patients. Nine and three regions of interest were positioned at the liver or spleen, respectively, to avoid macroscopic vessels. Median hepatic or splenic attenuation values were obtained. Hepatic attenuation minus splenic attenuation ( $CT_{L-S}$ ) less than 1 Hounsfield unit was considered fatty liver [25].

### **Statistical analysis**

Student's t-test was used to analyze differences in continuous variables between two groups. Categorical variables were compared with the Chi-squared or Fisher's exact test. Linear regression was performed to assess the relationship between two variables. Multiple logistic regression was performed to determine the independent factors associated with the presence of NAFLD. P-values <0.05 were considered significant. All analyses were performed with StatFlex version 6.0 for Windows (StatFlex, Osaka, Japan).

## **RESULTS**

Fatty liver was detected in 110 of 437 patients (25.2%). It was significantly associated with male gender ( $P = 0.005$ ), younger age ( $p < 0.001$ ), greater height ( $p < 0.001$ ) and weight ( $p < 0.001$ ), higher body mass index (BMI) ( $p < 0.001$ ), blood parameters [higher white blood cell counts ( $P = 0.021$ ) and higher hemoglobin ( $p < 0.001$ )], altered liver function tests [higher albumin ( $p < 0.001$ ), higher total bilirubin ( $P = 0.015$ ), aspartate

aminotransferase ( $P = 0.014$ ), alanine aminotransferase ( $p < 0.001$ ), and gamma glutamyl transpeptidase ( $p < 0.001$ ), kidney function [lower creatinine ( $P = 0.012$ ) and higher estimated glomerular filtration rate (eGFR) ( $P = 0.004$ )], metabolic status [higher total cholesterol ( $P = 0.012$ ), lower high density lipoprotein (HDL) cholesterol ( $P = 0.039$ ), higher triglycerides ( $p < 0.001$ ), and higher low density lipoprotein (LDL) cholesterol ( $P = 0.001$ )], a lower fibrosis-4 (FIB-4) index ( $P = 0.005$ ), and non-hypertensive ( $P = 0.013$ ) and non-dialysis patients ( $P = 0.003$ ) (Table 1). Fatty liver also tended to be associated with higher HbA1c ( $P = 0.066$ ).

Multivariate logistic regression was used to elucidate the independent factors associated with fatty liver. Gender, age, BMI, HbA1c, white blood cell count, hemoglobin, albumin, eGFR, total cholesterol, HDL cholesterol, triglyceride, LDL cholesterol, hypertension, and dialysis therapy were analyzed. Age ( $p < 0.001$ ), hemoglobin level ( $p < 0.001$ ), HDL cholesterol level ( $P = 0.002$ ), and absence of dialysis ( $P = 0.035$ ) were independent factors associated with fatty liver (Table 2).

The patients were stratified according to age, hemoglobin level, and HDL cholesterol level, and the association with fatty liver was assessed. The prevalence of fatty liver significantly decreased with increasing age ( $p < 0.001$ ) (Figure 2a), significantly increased as hemoglobin levels increased ( $p < 0.001$ ) (Figure 2b), and was significantly higher in patients with HDL cholesterol  $<70$  mg/dL (29.9%) than patients with HDL cholesterol  $\geq 70$  mg/dL (13.8%) ( $P = 0.012$ ) (Figure 2c).

## **DISCUSSION**

The prevalence of NAFLD in patients with T2DM detected by CT was 25.2%. NAFLD was associated with younger age, higher hemoglobin levels, lower HDL cholesterol levels, and the absence of dialysis.

A previous meta-analysis found that the prevalence of NAFLD in patients with T2DM was 56% with US or proton magnetic resonance spectroscopy [4]. The prevalence of NAFLD in patients with T2DM detected by CT was 10% in the United States [8], 22% in Turkey [9], and 27% in Japan [10]. Lee *et al* reported that the sensitivity of US (92%) for



detecting more than 30% hepatic steatosis was higher than CT (64%) [26]. The prevalence of NAFLD in this study is comparable to the reported prevalence in patients with T2DM diagnosed by CT in Japan. In a preliminary study of 179 subjects who attended health screening in our hospital (120 males and 59 females;  $53.7 \pm 10.8$  years), CT detected fatty liver in 40 (22%). Thus, the prevalence of NAFLD in T2DM is higher than in the general population considering older age.

The prevalence of NAFLD significantly decreased with increasing age in this study. In contrast, Targher *et al* reported that the prevalence of NAFLD detected by US in patients with T2DM increased with age; 65% among patients aged 40 to 59 and 75% among those aged 60 and older [14]. The difference in this study compared to Targher's regarding the association of NAFLD and age may be attributed to the difference in the mean age of the participants (74 vs. 64 years, respectively). By combining Targher's results and this study, it is suggested that the prevalence of NAFLD increases with age until about 60 years (as in Targher's study) and then decreases with age, as shown in this study. An "inverted U curve," in which the prevalence of NAFLD reaches a peak in late adulthood and decreases afterward has been reported in the general population [27-29]. However, a meta-analysis observed a consistent increase in the NAFLD prevalence across all age groups [30].

Poor nutritional status is more common in older people [31]. Age-related changes in appetite, health problems, and social problems predispose older adults to less food intake. Poor nutritional status in older people is also common in patients with T2DM [32]. The decreasing prevalence of NAFLD with age shown in the present study may be attributed to poor nutritional status. Age significantly negatively correlated with BMI in this study ( $r = -0.32$ ,  $p < 0.001$ , data not shown). A high BMI is associated with NAFLD [28] and a low BMI is associated with poor nutritional status [33]. BMI was associated with NAFLD in this study by univariate analysis but not by multivariate analysis.

In this study, lower hemoglobin values were associated with a lower prevalence of NAFLD. Anemia is common in the elderly and an indicator of poor nutritional status [34]. Therefore, the lower prevalence of NAFLD in patients with lower hemoglobin

values may be attributed to the poor nutritional status of these patients. Hemoglobin values also significantly positively correlated with BMI ( $r = 0.21$ ,  $p < 0.001$ , data not shown).

Higher HDL cholesterol values were associated with a lower prevalence of NAFLD. This association between HDL cholesterol and NAFLD has previously been reported [28].

Dialysis treatment was associated with a lower prevalence of NAFLD. Diabetic nephropathy affects approximately 25% of patients with T2DM and is the leading cause of renal failure. Two studies reported that NAFLD is inversely associated with nephropathy in patients with T2DM, similar to this study [20, 21]; however, some studies have reported a positive or no association [18, 19, 22]. The difference in the studied populations may cause this discrepancy. When the study population was not limited to patients with T2DM, a meta-analysis showed a positive association between NAFLD and chronic kidney disease [35]. However, in patients with T2DM, this association is still ambiguous.

This study did not find an association between NAFLD and cardiovascular disease. This association has been previously reported [7, 9, 10, 14]; however, other studies deny it [8, 15-17]. Higher HDL cholesterol values associated with a lower prevalence of NAFLD in this study reduce the risk of cardiovascular disease [36]. Thus a follow-up study may reveal an association between NAFLD and cardiovascular disease.

In this study, the number of patients with cirrhosis or hepatocellular carcinoma (HCC) was small, and cirrhosis and HCC were not associated with NAFLD. In a follow-up study of patients with T2DM (mean, 10.9 years), Adams *et al* reported that 5 of 116 patients with NAFLD and none of 221 patients without NAFLD died from liver-related causes [11]. Dunn *et al* reported that NAFLD was not associated with liver-related outcomes (transplant, HCC, or encephalopathy) in patients with T2DM in a five-year retrospective cohort study [8]. Further studies are needed to assess these associations.

There are three limitations to this study. First, it is a cross-sectional study. There may be a question of whether NAFLD decreases with age. Poor nutritional status in older



people may be one reason why NAFLD decreases with age. It is also possible that we assessed a certain subpopulation of T2DM with a low risk of NAFLD and survival until older age, while the patients with a high risk of NAFLD dropped out until older age because of complications. Thus, the temporal association of NAFLD with the factors assessed in this study has to be clarified by prospective cohort studies. Second, fatty liver was diagnosed by CT in this study. The sensitivity of MRI, US, and CT for detecting a fatty liver of 5% or higher is 77%–80%, 53%–62%, and 50% compared with liver biopsy [37]. However, a liver biopsy is invasive and has a risk of severe complications. Thus noninvasive modalities, such as US, CT, and MRI, have been commonly used to detect fatty liver. MRI is expensive and scarce. The disadvantage of US is its subjective nature. The high liver iron content increases CT Hounsfield units and may obliterate the diagnosis of fatty liver. However, CT is widely available in Japan, and the diagnosis is objective. Thus, CT is a promising modality for diagnosing fatty liver. Third, the present study was performed in a single hospital. The prevalence found has to be reevaluated in multicenter studies.

## **CONCLUSION**

The prevalence of NAFLD in patients with T2DM detected by CT was 25.2%. NAFLD was associated with age, hemoglobin level, HDL cholesterol level, and the absence of dialysis treatment.

## **ARTICLE HIGHLIGHTS**

### ***Research background***

Type 2 diabetes mellitus (T2DM) is an established risk factor for the development of nonalcoholic fatty liver disease (NAFLD). Both synergistically increase adverse outcomes.

### ***Research motivation***

The prevalence of NAFLD assessed by computed tomography (CT) was reported only in a few studies. The clinical correlates of NAFLD are often ambiguous.

### ***Research objectives***

To determine the prevalence and clinical correlates of NAFLD assessed by CT in a large cohort of T2DM patients.

### ***Research methods***

Four hundred thirty-seven participants with T2DM who consulted at Meijo Hospital from April 2019 to September 2020 and underwent CT were assessed.

### ***Research results***

The prevalence of NAFLD as detected by CT was 25.2% in T2DM patients, and NAFLD was associated with a younger age, higher of hemoglobin levels, lower high density lipoprotein cholesterol levels, and absence of dialysis treatment.

### ***Research conclusions***

The <sup>2</sup> prevalence of NAFLD in T2DM is higher than in the general population considering older age and decreases with age.

### ***Research perspectives***

The association of NAFLD with age has to be clarified by prospective cohort studies.

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