

RESEARCH ARTICLE

Non-alcoholic Fatty Liver Disease (NAFLD) and Significant Hepatic Fibrosis Defined by Non-invasive Assessment in Patients with Type 2 Diabetes

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD), the most common liver problem in diabetes, is a risk factor for liver cancer. Diabetes, high body mass index (BMI) and old age can all contribute to NAFLD progression. Transient elastography (TE) is used for non-invasive fibrosis assessment. **Objectives:** To identify the prevalence of NAFLD and significant hepatic fibrosis in diabetic patients and to assess associated factors. **Materials and Methods:** One hundred and forty-one diabetic and 60 normal subjects were screened. Fatty liver was diagnosed when increased hepatic echogenicity and vascular blunting were detected by ultrasonography. Liver stiffness measurement (LSM) representing hepatic fibrosis was assessed by TE. $LSM \geq 7$ kPa was used to define significant hepatic fibrosis. **Results:** Four cases were excluded due to positive hepatitis B viral markers and failed TE. Diabetic patients had higher BMI, systolic blood pressure, waist circumference and fasting glucose levels than normal subjects. Fatty liver was diagnosed in 82 (60.7%) diabetic patients but in none of the normal group. BMI (OR: 1.31; 95% CI: 1.02-1.69; $p=0.038$) and alanine aminotransferase (ALT) (OR: 1.14; 95% CI: 1.05-1.23; $p=0.002$) were associated with NAFLD. Diabetic patients with NAFLD had higher LSM than those without [5.99 (2.4) vs 4.76 (2.7) kPa, $p=0.005$]. Significant hepatic fibrosis was more common in diabetic patients than in normal subjects [22 (16.1%) vs 1 (1.7%), $p=0.002$]. Aspartate aminotransferase (AST) (OR: 1.24; 95% CI: 1.07-1.42; $p=0.003$) was associated with significant hepatic fibrosis. **Conclusions:** Sixty and sixteen percent of diabetic patients were found to have NAFLD and significant hepatic fibrosis. High BMI and ALT levels are the predictors of NAFLD, and elevated AST level is associated with significant hepatic fibrosis.

Keywords: Diabetes - Non-alcoholic fatty liver disease (NAFLD) - hepatic fibrosis - transient elastography

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Introduction

Alcohol, hepatitis B and hepatitis C viral infection are common causes of cirrhosis and hepatocellular carcinoma in Thailand and Asian countries (Gao et al., 2012; Somboon et al., 2014). High body mass index (BMI) and type 2 diabetes mellitus are associated with an increased risk of death from cancer and ischemic heart diseases (Fujino, 2007; Chiou et al., 2011). In 2012, more than three hundred and seventy-one million people have diabetes with an increased prevalence of diabetes worldwide. Liver disease is the third most common cause of death in diabetic patients (Tolman et al., 2007). Cryptogenic cirrhosis has become one of the most frequent indications of liver transplantation in the U.S. The spectrum of liver abnormalities in patients with type 2 diabetes includes abnormal liver enzymes, non-alcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma and acute liver failure (Tolman et al., 2007). Mild elevation of serum alanine aminotransferase (ALT) is seen in 2-24% of

diabetic patients (Trombetta et al., 2005). NAFLD is by far the most common cause of a mild elevation of serum ALT in type 2 diabetes (Harris et al., 1998). NAFLD consists of a spectrum of chronic liver diseases from simple steatosis to non-alcoholic steatohepatitis (NASH) and advanced fibrosis (Teli et al., 1995).

Evidence from several longitudinal follow-up studies has confirmed that NASH can progress to advanced fibrosis and cirrhosis (Teli et al., 1995). NAFLD alone or NAFLD coexisting with hepatitis B or C viral infection can progress to cirrhosis (Poortahmasebi et al., 2014). Diabetes, body mass index (BMI), and age are predictors of the presence of advanced fibrosis in NASH (Angulo et al., 2007). Liver biopsy remains the gold standard tool to assess for fibrotic staging of the liver. However, the benefit of liver biopsy comes with the risks of major complications (1-3%) and mortality (0.01%) in patients undergoing liver biopsy (Bravo et al., 2001). Liver stiffness measurement (LSM) performed by transient elastography (TE) (Fibroscan®, Echogen, Paris), which

uses ultrasound-based technology for quantitative assessment of hepatic fibrosis, has been studied in chronic liver diseases including NAFLD (Wong et al., 2010).

In this study, we aimed to evaluate for the prevalence of NAFLD and significant hepatic fibrosis in patients with diabetes, and to identify the risk factors of NAFLD and significant hepatic fibrosis in these patients.

Materials and Methods

A cross-sectional study was performed at the liver unit, Ramathibodi Hospital, Mahidol University between October 1st 2011 and September 30th 2012. The study protocol was approved by the Hospital Ethics Committee, and it was carried out according to the Helsinki Declaration guideline. Written informed consent was obtained prior to recruitment. Type 2 diabetic patients and normal subjects were enrolled. Subjects with known history of liver diseases or cirrhosis, regular alcohol drinking more than 20 mg per day and current herbal substance use were excluded. Normal subjects were healthy volunteers with no medical conditions including liver diseases, no history of regular alcohol drinking, normal biochemical tests and negative hepatitis B surface antigen (HBsAg) and anti-hepatitis C viral antibody (anti-HCV ab). Demographic data were collected. Body mass index (BMI) was calculated from weight (kg) divided by height² (m). BMI of 23.0 to 24.9 kg/m² for overweight and ≥ 25.0 kg/m² for obesity in adult Asians as proposed by the World Health Organization, International Association for the Study of Obesity and International Obesity Task Force in 2000 were employed in this study (WHO, 2000; Alberti et al., 2005). BMI of 25-29.9 kg/m² and ≥ 30 kg/m² are consistent with obesity class 1 and 2 (WHO, 2000).

Waist circumference was measured at the level of umbilicus in the upright position. Fasting serum samples were taken for hematological and biochemical testing which included complete blood count, liver function test, serum glucose, glycated hemoglobin (HbA1C), lipid panel, hepatitis B and C viral markers. The definition of metabolic syndrome followed the criteria proposed by the International Diabetes Federation (IDF) (Alberti et al., 2005). The IDF criteria require central obesity (: WC ≥ 90 cm in men and ≥ 80 cm in women for Asians) plus 2 of the following 4 criteria: triglyceride ≥ 150 mg/dl, HDL cholesterol < 40 mg/dl in men and < 50 mg/dl in women, fasting plasma glucose ≥ 100 mg/dl or presence of type 2 diabetes, and blood pressure $\geq 130/85$ mmHg or on antihypertensive medication (Alberti et al., 2005).

Abdominal ultrasonography

Diabetic patients and normal subjects underwent abdominal ultrasonography (HITACHI, Japan) using a convex 3.5 MHz probe for evaluation of fatty liver. The diagnosis of fatty liver was made based on increased liver brightness with presence of sonographic contrasted appearance between liver and kidney parenchyma, high posterior attenuation, reduced vessel wall echogenicity and poorly delineated diaphragm (Hamer et al., 2006).

Transient elastography (TE)

TE was performed by a single experienced operator using FibroScan (Echosens, Paris, France) with a medium (M) probe operating at a frequency of 3.5 MHz. A successful TE examination was defined as ≥ 10 valid measurements with $> 60\%$ success rate and an interquartile range (IQR) to median ratio of ≤ 0.30 (Jung and Kim, 2012). The median value of 10 valid measurements was considered to be the liver stiffness measurement (LSM) which was reported as kPa. Significant hepatic fibrosis [or fibrotic (F) stage ≥ 2 diagnosed by liver biopsy] was defined as LSM ≥ 7 kPa based on the result of a study of LSM using transient elastography in patients with NAFLD (Wong et al., 2010).

Statistical analysis

Descriptive data were expressed as number (%) for discrete variables, mean (SD) or median (range) for continuous variables. Statistical analysis of continuous variables was performed by Student's t-test or non-parametric test whatever appropriate. Chi-square test or Fisher exact test was used for analysis of discrete variables. P-value < 0.05 was considered statistically significant. For variables with P-value < 0.20 from univariate analysis, they were analyzed further with multivariate analysis using logistic regression or multiple regression analysis as appropriate.

Results

A total of 141 patients with type 2 diabetes and 60 normal subjects were screened. Four diabetic patients were ineligible for recruitment because of positive HBsAg in 3 patients and failed TE in 1 patient.

Diabetic patients vs normal subjects

Demographic data of 137 diabetic patients and 60 normal subjects are shown in Table 1. Diabetic patients were older than normal subjects with mean age (SD) of 60.5 (9.8) vs 39.8 (15.2) years ($p=0.00$). Mean BMI and waist circumference were also higher in diabetic patients [26.0 (4.5) vs 23.0 (3.3) kg/m² ($p=0.00$), and 89.1 (11.4) vs 80.1 (9.20) cm ($p=0.00$)]. Among 137 diabetic patients, 29 (21.2%), 54 (39.4%), 22 (16.1%) patients were classified as overweight, obesity class 1 and obesity class 2 according to BMI classification of adult Asians (WHO, 2000). The results of ultrasonography of two diabetic patients were missing. Eighty-two of 135 diabetic patients (60.7%) had fatty liver based on ultrasonographic criteria while none in normal group showed evidence of fatty liver from ultrasonography. Mean LSM was significantly higher in diabetic patients than normal subjects (5.52 vs 4.16 kPa, $p=0.00$). Twenty-two patients (16.1%) with diabetes had significant hepatic fibrosis, which was defined by LSM ≥ 7.0 kPa, comparing to 1 patient (1.7%) in normal group ($p=0.002$).

NAFLD in diabetic patients

Out of 135 diabetic patients, ultrasonographic examination revealed fatty liver in 82 patients (60.7%) and normal liver in 53 patients (39.3%). The prevalence of fatty liver was progressively increased from 17.2% in normal

weight patients to 58.6% in overweight patients, 77.8% in obesity class 1 and 85.7% in obesity class 2 diabetic patients (p=0.00). Seventy-two patients (60.5%) out of 119 patients had metabolic syndrome by the IDF definition (Alberti et al., 2005). NAFLD was more commonly seen in diabetic patients with metabolic syndrome than in those without metabolic syndrome [59 (76.6%) vs 13 (31.0%), p=0.00]. The characteristics of diabetic patients with and without fatty liver are shown in Table 2. Univariate analysis revealed that diastolic blood pressure (DBP), BMI, WC, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride (TRIG) levels were significantly higher in diabetic patients with NAFLD than those without NAFLD. Factors with p<0.20 (age, DBP, BMI, WC, AST, ALT, TRIG) were analyzed further with

logistic regression analysis. BMI (OR: 1.31; 95%CI: 1.02-1.69; p=0.038) and ALT (OR: 1.14; 95%CI: 1.05-1.23; p=0.002) were independently associated with the presence of NAFLD in diabetic patients.

Significant hepatic fibrosis in diabetic patients

LSM, which was used to assess for the severity of hepatic fibrosis, in diabetic patients was significantly higher than in normal subjects [5.52 (2.4) vs 4.16 (0.9)]. TE revealed that twenty-two diabetic patients (16.1%) had fibrotic stages of at least significant fibrosis. The characteristics of diabetic patients with or without significant fibrosis are shown in Table 3. Univariate analysis revealed that AST, ALT levels and the diagnosis of fatty liver by ultrasound were significantly related to

Table 1. Demographic Data of Diabetic Patients and Normal Subjects

	Normal subjects	Diabetic patients	p-value
Number	60	137	
Age* (year)	39.82 (15.2)	60.47 (9.8)	0.00
Sex			0.43
Female	38 (63.3%)	77 (56.2%)	
Male	22 (36.7%)	60 (43.8%)	
Blood pressure*(mmHg)			
Systolic blood pressure	113.5 (15.4)	127.3 (15.5)	0.00
Diastolic blood pressure	70.9 (11.0)	73.5 (10.7)	0.18
Body mass index*(kg/m ²)	23.0 (3.3)	26.0 (4.5)	0.00
Waist circumference*(cm)	80.1 (9.2)	89.1 (11.4)	0.00
Aspartate aminotransferase*(u/l)	24.5 (31.1)	25.3 (10.9)	0.79
Alanine aminotransferase*(u/l)	38.0 (30.0)	41.7 (19.3)	0.85
Cholesterol*# (mg/dl)	200.6 (38.1)	185.8 (38.5)	0.02
Triglyceride*# (mg/dl)	117.0 (91.4)	138.0 (71.4)	0.16
Fasting glucose*(mg/dl)	90.5 (7.8)	135.5 (41.2)	0.00
Hemoglobin A1c*(%)	5.8 (0.5)	7.3 (1.4)	0.00
Liver stiffness measurement*(kPa)	4.2 (0.9)	5.5 (2.4)	0.00
Interquartile range (IQR)	0.5 (0.4)	0.8 (0.9)	0.02
Interquartile range (IQR)/median	0.1 (0.1)	0.1 (0.1)	0.52
Liver stiffness measurement ≥7.0 kPa	1 (1.70%)	22 (16.1%)	0.002
Fatty liver by ultrasonography	0 (0%)	82 (60.7%)	0.00

*mean (SD); #under treatment

Table 2. Characteristics of Diabetic Patients with and without Non-alcoholic Fatty Liver Disease (NAFLD)

	Diabetic patients without NAFLD	Diabetic patients with NAFLD	p-value
Number (%)	53 (39.3)	82 (60.7)	
Age* (year)	62.1 (11.3)	59.4 (8.7)	0.15
Sex			0.32
Female	28 (36.8 %)	48 (63.2%)	
Male	25 (42.4%)	34 (57.6%)	
Blood pressure*(mmHg)			
Systolic blood pressure	126.5 (17.7)	128.0 (14.1)	0.62
Diastolic blood pressure	70.9 (10.7)	75.2 (10.5)	0.03
Body mass index*(kg/m ²)	23.4 (3.8)	27.6 (4.1)	0.00
Waist circumference*(cm)	83.0 (10.6)	92.9 (9.8)	0.00
Aspartate aminotransferase*(u/l)	22.4 (7.7)	27.3 (12.3)	0.007
Alanine aminotransferase*(u/l)	32.6 (9.0)	47.7 (21.9)	0.00
Cholesterol*# (mg/dl)	189.8 (38.7)	183.9 (38.0)	0.40
Triglyceride*# (mg/dl)	107.9 (52.7)	161.5 (75.7)	0.00
Fasting glucose*(mg/dl)	132.5 (34.9)	137.6 (45.3)	0.47
Hemoglobin A1c*(%)	7.4 (1.5)	7.2 (1.3)	0.59
Liver stiffness measurement*(kPa)	4.8 (2.7)	6.0 (2.0)	0.005
Interquartile range (IQR)	0.60 (0.3)	0.85 (1.1)	0.07
Interquartile range (IQR)/median	0.13 (0.06)	0.14 (0.17)	0.61
Liver stiffness measurement ≥7.0 kPa	3 (5.7%)	18 (22.0%)	0.008

*mean (SD); #under treatment

Table 3. Characteristics of Diabetic Patients with and without Significant Fibrosis by Liver Stiffness Measurement

	Diabetic patients without significant fibrosis	Diabetic patients with significant fibrosis	p-value
Number (%)	115 (83.9)	22 (16.1)	
Age* (year)	59.9 (9.9)	63.8 (9.2)	0.10
Sex			0.52
Female	65 (84.4 %)	12 (15.6%)	
Male	50 (83.3%)	10 (16.7%)	
Blood pressure*(mmHg)			
Systolic blood pressure	126.7 (15.7)	130.6 (14.8)	0.27
Diastolic blood pressure	73.6 (10.5)	73.1 (11.9)	0.88
Body mass index*(kg/m ²)	25.7 (4.4)	27.6 (5.0)	0.09
Waist circumference*(cm)	88.1 (10.5)	94.2 (14.4)	0.07
Aspartate aminotransferase*(u/l)	23.1 (7.9)	36.5 (16.2)	0.001
Alanine aminotransferase*(u/l)	38.6 (14.7)	57.6 (30.3)	0.008
Cholesterol*# (mg/dl)	186.5 (38.3)	181.5 (40.9)	0.64
Triglyceride*# (mg/dl)	139.8 (75.7)	129.3 (45.0)	0.42
Fasting glucose*(mg/dl)	132.5 (35.8)	151.4 (60.9)	0.17
Hemoglobin A1c*(%)	7.2 (1.3)	7.6 (1.8)	0.38
Fatty liver by ultrasonography	64 (56.1%)	18 (85.7%)	0.008

*mean (SD); #under treatment

the presence of significant fibrosis. Factors with $p < 0.20$ (age, BMI, WC, AST, ALT, fasting glucose, presence of NAFLD) were analyzed further with logistic regression analysis. Only AST level was independently associated with significant hepatic fibrosis (OR: 1.24; 95%CI: 1.07-1.42; $p = 0.003$). Eight patients (5.93%) had $LSM \geq 8.7$ kPa which was suggested to be the LSM cutoff of advanced fibrosis in NAFLD patients (Wong et al., 2010). NAFLD was detected in 6 patients (75%) who had $LSM \geq 8.7$ kPa. One patient had LSM of 21.8 kPa. Liver biopsy was done in this case and cirrhosis was confirmed. NAFLD was likely to be the cause of advanced fibrosis or cirrhosis in these 8 patients, except for 1 case who had history of regular alcohol drinking for 30 years and quit alcohol drinking five years ago.

Factors related to liver stiffness measurement (LSM)

From univariate analysis of the diabetic patient data, it revealed that BMI, WC, AST, ALT and presence of fatty liver by ultrasonography were significantly related to LSM. From multiple regression analysis, AST was independently associated with LSM (coefficient of 0.1, standard error of 0.03 and p-value of 0.0005).

Discussion

The clinical entity of NAFLD ranges from benign simple steatosis to non-alcoholic steatohepatitis (NASH). Hepatic fibrosis is frequently associated with NASH, and about 10% of NASH patients can progress to cirrhosis (Teli et al., 1995). The risk of hepatocellular carcinoma increases after the development of liver cirrhosis (Teli et al., 1995). Patients with diabetes, similar to those with dyslipidemia, hypertension, or other metabolic syndrome, are at risk of NAFLD (Cusi, 2009). Hyperinsulinemia and hyperglycemia in diabetes promote lipogenesis by upregulating hepatic sterol regulatory element binding protein 1c (SREBP1c) and carbohydrate regulatory element binding protein (ChREBP) activity (Adiels et al., 2008; Cusi, 2009). Insulin-resistant adipose tissue in

diabetes is another factor that promotes the development of steatosis (Adiels et al., 2008; Cusi, 2009). In addition, de novo lipogenesis stimulated by high carbohydrate and fructose intake lead to the activation of hepatic inflammatory pathway (Adiels et al., 2008; Cusi, 2009). Previous studies have confirmed that NAFLD patients with diabetes are at a higher risk for the progression to advanced fibrosis and cirrhosis than those without diabetes (Teli et al., 1995; Younossi et al., 2004).

Diabetes is seen in 18-33% of patients with NAFLD. On the contrary, 49-69.4% of patients with diabetes are reportedly to have NAFLD (Younossi et al., 2004; Trombetta et al., 2005; Tolman et al., 2007; Cusi, 2009; Leite et al., 2009). NAFLD is the most common cause of serum ALT elevation in diabetes (Younossi et al., 2004). In this study, the prevalence of NAFLD in diabetic patients at out-patient clinics of our hospital, which is a tertiary care hospital, was about 60%. The reported prevalence of NAFLD in diabetes varies according to the methods used to evaluate the liver. The gold standard for steatosis and fibrosis assessment is liver biopsy (Schwenzer et al., 2009; Hernaez et al., 2011). However, liver biopsy is an invasive technique which can yield minimal risks of morbidity and mortality. MR spectroscopy (MRS) is a non-invasive, highly accurate technique for fat quantification, because it measures proton signal from hepatocyte triglyceride stores directly (Schwenzer et al., 2009). However, a high cost of MRS technique, which is not universal accessibility and a long period of time required for examination are important obstacles of MRS study. Ultrasonography is inexpensive and widely available. It has 60-94% sensitivity and 66-97% specificity for grading of hepatic steatosis (Schwenzer et al., 2009; Hernaez et al., 2011). However, ultrasonography detects fat in the liver only when there is hepatic fat accumulation more than 33% (Schwenzer et al., 2009; Hernaez et al., 2011). We showed that the predictive factors of having NAFLD identified by ultrasonography in diabetes patients were BMI (OR: 1.31; 95%CI: 1.02-1.69; $p = 0.038$) and ALT level (OR: 1.14; 95%CI: 1.05-1.23; $p = 0.002$). The prevalence of

NAFLD was more commonly seen in diabetic patients with obesity class 1 and 2, and in diabetic patients with fulfilled criteria of metabolic syndrome. The result of this study is consistent with some conclusion from a previous report which showed the association of abdominal obesity, high ALT level and hypertriglyceridemia with the presence of NAFLD in diabetes patients (Leite et al., 2009).

Though liver biopsy is a gold standard for fibrosis assessment in chronic liver disease and NAFLD, it cannot be done routinely due to the risks of complications and death. Noninvasive fibrosis assessment includes liver enzymes, serum biomarkers and transient elastography (TE) which has been studied in chronic liver diseases including NAFLD. Serum biomarkers such as CK18 levels and CK18 have been developed to replace liver biopsy. CK18, which is a marker of necrosis has been used for differentiating NASH from simple steatosis (Jacqueminet et al., 2008). From a study of CK18 in diabetic patients, 4.3% of patients were found to have advanced fibrosis (Jacqueminet et al., 2008). The results of LSM study in various chronic liver diseases using TE have been reported. TE is more accurate than biologic markers for fibrosis assessment in NAFLD (Wong et al., 2010). The best cutoffs of LSM in NAFLD for F2 staging or greater, F3 staging or greater and F4 are 7.0, 8.7 and 10.3 kPa, respectively (Wong et al., 2010). By using the LSM cutoffs from the previous study (Wong et al., 2010), we found that 22 diabetic patients (16.1%) had significant hepatic fibrosis (LSM \geq 7 kPa) and 8 patients (5.84%) had advanced fibrosis or higher (LSM \geq 8.7 kPa). In this study, the prevalence of diabetic patients with significant fibrosis as assessed by TE was lower than the result from a recent study of diabetic patients in Australia (Casey et al., 2012). In that report, 26/74 patients (35%) had LSM \geq 7.65 kPa, with biopsy-confirmed significant fibrosis in 12/16 patients and cirrhosis in 2 patients (Casey et al., 2012). The difference in prevalence of significant fibrosis in the diabetic Australian study (Casey et al., 2012) and ours may be explained by the higher BMI and WC of the Australian patients when compared to our patients [(32.8 vs 25.97 kg/m²) and (111.3 vs 89.08 cm)]. Likewise, higher mean LSM and prevalence of NAFLD were seen in Australian patients than in our Thai patients [(8.3 vs 5.52 kPa) and (70 vs.60.7%)]. Although AST, ALT level and NAFLD were found to be significant factors in univariate analysis, similar to the results from the previous study (Casey et al., 2012), AST level alone was associated with significant fibrosis (OR: 1.24; 95%CI: 1.07-1.42; p=0.003). Furthermore, AST level was found to be an independent factor relating to LSM from multiple regression analysis.

Lack of liver biopsy in every case to confirm hepatic fibrotic staging in this study is the crucial limitation of our report. However, we avoided the inaccuracy of TE measurement by following the standard protocol for TE performance issued by Echosen company, and the TE machine has had regularly inspected and calibrated. About 60 percent and 16 percent of diabetic patients had NAFLD and significant hepatic fibrosis by noninvasive assessment. High BMI and elevated ALT level were the predictors of NAFLD in diabetic patients. The predictive

factor of significant fibrosis in diabetic patients was AST level. However, further studies are required to confirm the results of this study.

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