

THE ASSOCIATION BETWEEN RISK FACTORS AND ULTRASOUND-BASED GRADES OF NON-ALCOHOLIC FATTY LIVER DISEASE IN TYPE-2 DIABETES PATIENTS

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THE ASSOCIATION BETWEEN RISK FACTORS AND ULTRASOUND-BASED
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PATIENTS

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) has become more common as the cause of cirrhosis and liver cancer. The liver disease is highly prevalent in people with type-2 diabetes. Indonesia is not spared from the global epidemic of type-2 diabetes. The ultrasound examination is clinically easy-to-use, economical and non-invasive as a tool to detect NAFLD, compared to the gold standard, liver biopsy. To date, there has been no study in Indonesia to link risk factors and ultrasound-based severity grading of NAFLD. **Aim:** To understand the association between risk factors and ultrasound-based grades of NAFLD in patients with type-2 diabetes. **Method:** The present study was an observational study with a cross-sectional design (May-October 2018) that involved 82 type-2 diabetes outpatients of the internal medicine clinic in the Gotong Royong Hospital (Surabaya, Indonesia). The risk factors assessed were gender, age, diabetes duration, obesity (anthropometric measurement: body mass index/ BMI, waist circumference and waist-to-hip ratio), glycemic control (hemoglobin A1c/ HbA1c level) and dyslipidemia (lipid profile: total cholesterol, low-density lipoprotein/ LDL, high-density lipoprotein/ HDL and triglyceride). The ultrasound-based grades of NAFLD consisted of grade 0 (no NAFLD), grade 1 (increased liver echogenicity with normal images of intrahepatic vessel lines and diaphragm), grade 2 (blurred image of intrahepatic vessel lines) and grade 3 (blurred images of intrahepatic vessel lines and diaphragm). Statistical p-value was significant at ≤ 0.05 . **Results:** Seventy-eight subjects (95,1%) had NAFLD. The ultrasound-based NAFLD grades were significantly different across age groups (Kruskal-Wallis) but the Spearman's rank correlation test result was not significant. Body mass index and total cholesterol were positively correlated ($r = 0.390$ and 0.237 , respectively) with the NAFLD grades. **Conclusion:** Higher BMI and total cholesterol are associated with increased ultrasound-based NAFLD grades.

Keywords: Diabetes Tipe 2, Non-Alcoholic Fatty Liver Disease, Risk Factors, Ultrasonography

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ABSTRAK

Latar belakang: Penyakit perlemakan hati non-alkoholik menjadi lebih sering sebagai penyebab sirosis dan kanker hati. Penyakit hati ini memiliki prevalensi tinggi pada orang yang menderita diabetes tipe 2. Indonesia juga memiliki prevalensi penyakit diabetes tipe 2 yang tinggi seperti di dunia. Pemeriksaan ultrasonografi mudah secara klinis, murah dan tidak invasif untuk mendeteksi penyakit perlemakan hati non-alkoholik, dibandingkan biopsi hati sebagai standar emas. Sampai saat ini, belum ada studi di Indonesia yang menghubungkan faktor risiko dan derajat keparahan penyakit perlemakan hati non-alkoholik berdasarkan ultrasonografi.

Tujuan: Untuk memahami hubungan antara faktor risiko dan derajat penyakit perlemakan hati non-alkoholik berdasarkan ultrasonografi pada pasien diabetes tipe 2. **Metode:** Studi ini adalah

suatu studi observasional dengan desain potong lintang (Mei-Oktober 2018) yang melibatkan 82 pasien diabetes tipe 2 di poli penyakit dalam, Rumah Sakit Gotong Royong (Surabaya, Indonesia).

Faktor risiko yang dinilai adalah jenis kelamin, usia, lama menderita diabetes, obesitas (indeks massa tubuh, lingkaran pinggang dan rasio lingkaran pinggang-pinggul), kontrol glikemik (kadar HbA1c) dan dilipidemia (profil lipid: kolesterol total, LDL, HDL dan trigliserida). Derajat penyakit perlemakan hati non-alkoholik berdasarkan ultrasonografi terdiri dari derajat 0 (tidak ada penyakit perlemakan hati non-alkoholik), derajat 1 (peningkatan ekogenisitas hati dengan gambaran normal batas pembuluh darah intrahepatik dan diafragma), derajat 2 (pengaburan gambaran batas pembuluh darah intrahepatik) dan derajat 3 (pengaburan gambaran pembuluh darah intrahepatik dan diafragma). Nilai statistik p signifikan pada ≤ 0.05 .

Hasil: Tujuh puluh delapan (95,1%) subyek memiliki penyakit perlemakan hati non-alkoholik. Derajat penyakit perlemakan hati non-alkoholik berdasarkan ultrasonografi berbeda secara signifikan antar kelompok usia (Kruskal-Wallis) tetapi hasil tes korelasi Spearman tidak signifikan. Indeks massa tubuh ($r = 0.390$) dan kolesterol total ($r = 0.237$) berkorelasi secara positif dengan derajat NAFLD berdasarkan ultrasonografi. **Simpulan:** BMI dan kolesterol total yang lebih tinggi berhubungan dengan peningkatan derajat penyakit perlemakan hati non-alkoholik berdasarkan ultrasonografi.

Kata kunci: diabetes tipe 2, penyakit perlemakan hati non-alkoholik, faktor risiko, ultrasonografi

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INTRODUCTION

Non-alcoholic fatty liver disease is presently the world's most common chronic liver disease. Based on results from a recent meta-analysis study, the disease worldwide prevalence is 25% (1). Owing to the change from an agrarian to industrial society that promotes an unhealthy diet and lifestyle, the prevalence of the disease in Asia has stacked up against the global one (around 25%) (2). Although studies about NAFLD conducted in Indonesian communities are still few, a systematic review which gathered data from hospital-based research has mentioned that the prevalence of the disease in the country is 30% (3).

Non-alcoholic fatty liver disease is a chronic liver disease which histological hallmarks are similar to the alcohol-induced liver disease but the patients have no history of excessive alcohol intake. It encompasses a spectrum of liver disease that ranges from simple steatosis to non-alcoholic steatohepatitis (NASH). The latter is likely to develop into liver cirrhosis and subsequently liver cancer. The multiple-hit pathogenesis is hitherto widely accepted as how NAFLD is initiated and progresses to become NASH, cirrhosis and liver cancer. In the pathogenesis theory, multiple factors are attributed to disease progression. Insulin resistance is followed by hepatic fat accumulation that causes inflammation of liver tissue. Gut microbiota imbalance and

genetic predisposition are also factored in (4–6). A 2013 population-based study in the US revealed that NASH-related liver cancer was the second-highest cause of liver transplantation (7). Non-alcoholic fatty liver disease is also an independent risk factor for patients to develop cardiovascular diseases (8).

Diagnosis of NAFLD has been made in 70-75% of patients with diabetes mellitus. This is not surprising as insulin resistance is the key factor in the NAFLD pathogenesis (9,10). A 10-year cohort study that involved more than 500,000 people in China has shown that subjects with diabetes have higher risks to develop NAFLD (hazard ratio 1,07), cirrhosis (1,07) and liver cancer (1,04) than those without diabetes (11). About 9% of adults in the world suffered from diabetes mellitus in 2015 according to the data from The International Diabetes Federation and Asia as the most populous continent contributed the most to the number. It is noteworthy that Indonesia is the third most populous country in Asia. Type-2 diabetes which is lifestyle-related holds the majority (90%) (12).

Age, gender and other features of metabolic syndrome (obesity, diabetes, and dyslipidemia) are risk factors of NAFLD that have been studied in populations (13). A research paper published in 2017 showed that the prevalence of NAFLD among patients with type 2 diabetes that visited

Cipto Mangunkusumo Hospital in Jakarta was 45.2% and 25.0% of these patients had liver fibrosis based on the fibroscan examination. In the study, the only risk factor associated with the incidence of NAFLD (diagnosis based on the ultrasound examination) was body mass index (BMI) (14).

Liver biopsy remains the gold standard of NAFLD diagnosis with the severity determined histologically. However, the invasive nature of liver biopsy makes it the last resort of diagnostic means which is used in such cases that other etiologies of liver disease should be excluded in patients with NAFLD (4,8). Despite all the limitations, ultrasound is clinically easy-to-use and low-cost as a diagnostic tool to detect NAFLD which usually shows no symptoms in the patients. Some parameters of ultrasonographic findings have been used to grade the severity of NAFLD to match the histological grading counterpart (15,16).

Several points have become the reason to conduct this current research. To the best of our knowledge, no paper studies regarding the association between risk factors and ultrasound-based grades of NAFLD in Indonesia have been published. The management of modifiable risk factors such as obesity, glycemic control and dyslipidemia is imperative in the treatment of patients with NAFLD (8). We would like

to assess if ultrasound-based grading of NAFLD severity is relevant to the existence of risk factors in type-2 diabetes patients. Therefore, the aim of this study is to understand the association between risk factors and ultrasound-based grades of NAFLD in patients with type-2 diabetes.

SUBJECTS AND METHODS

The observational study had a cross-sectional design and was conducted for 6 months (May-October 2018). Research subjects who were enrolled in this study were outpatients (age 30-80 years) of the internal medicine clinic in Gotong Royong Hospital (Surabaya, Indonesia). Ninety-seven patients participated in the study but only 82 matched the criteria. The inclusion criteria were the agreement to participate voluntarily (patients filled and signed the informed consent) and the diagnosis of type-2 diabetes had been made at least 3 months before the study was conducted. Patients would be excluded from the study if they had hepatitis B/hepatitis C infection, non-NAFLD chronic liver diseases and anemia (hemoglobin level was lower than 12 g/dl for males and 11 g/dl for females, which would lower the Hemoglobin A1c level). Other exclusion criteria were regular alcohol consumption (weekly intake more than 140 g for males and 70 g for females) and long-term treatment with hepatotoxic agents (e.g. isoniazid for tuberculosis

patients). The study protocol had gained approval from The Ethics Committee of Widya Mandala Catholic University Surabaya Faculty of Medicine.

Patients filled a data form and signed the informed consent. Information on age, gender and duration of diabetes was taken from the data form. Ages of patients were categorised into 5 groups (30-39 years, 40-49 years, 50-59 years, 60-69 years and 70-80 years). Patients were divided into male and female groups based on the gender option in the form. Furthermore, there were two duration of diabetes categories, patients who had diabetes for < 5 years and \geq 5 years (14).

Anthropometric measurements (height and weight for BMI, waist circumference, and hip circumference) were taken afterwards. The BMI formula is weight in kilograms/ height in meters². Patients were divided into two groups based on the BMI, ideal BMI (<25) and overweight (\geq 25). For the waist circumference, patients were considered to have an ideal waist circumference if the circumference < 90 centimeters (cm) for males and < 85 cm for females. Patients were considered to have a high waist circumference if the circumference \geq 90 cm for males and \geq 85 cm for females. Another component of the anthropometry used for determining obesity was the waist-to-hip ratio which formula is

waist circumference divided by hip circumference. The ideal waist-to-hip ratio is < 0.9 for males and < 0.85 for females. The high ratio is \geq 0.9 for males and \geq 0.85 for females (17).

Venous blood was taken from the patients to check the HbA1c (device: Epithod 616) and lipid profile (device: Cobas C111; total cholesterol, LDL, HDL and triglyceride) levels after fasting for 8 hours (overnight). Patients were divided into two groups based on the HbA1c level, < 7% (good glycemic control) and \geq 7% (poor glycemic control) (18). The LDL level is considered normal if the level < 100 milligram (mg)/ deciliter (dl) and high if the level \geq 100 mg/ dl. The HDL level is considered normal if the level \geq 50 mg/ dl for males and \geq 40 mg/ dl for females; and low if the level < 50 mg/ dl for males and < 40 mg/ dl for females. The normal triglyceride level is < 150 mg/ dl and the high level is \geq 150 mg/ dl (19).

An abdominal ultrasound (device: GE, logic S7 expert) examination was performed by a competent radiologist. The ultrasound-based grades of NAFLD were as follows: (1) grade 0: normal which means there is no increase of the liver echogenicity, (2) grade 1: mild which means an increase of the liver echogenicity with normal diaphragm image and intrahepatic blood vessel lines, (3) grade 2:

moderate which means an increase of liver echogenicity with blurred intrahepatic blood vessel lines but the diaphragm image is still normal; and (4) grade 3: severe which means an increase of liver echogenicity with blurred diaphragm image and intrahepatic blood vessel lines (20).

Data were analysed statistically using SPSS (Statistical Package for Social Sciences) version 25. The independent variables were age, gender, duration of diabetes, BMI, waist circumference, waist-to-hip ratio, HbA1c, cholesterol, LDL, HDL, and triglyceride. The dependent variable was the ultrasound-based NAFLD grade. The comparative test used for the independent variable that consisted of two groups was the Mann-Whitney test and the Kruskal-Wallis test was for the independent variable with more than two groups. If the comparative test result was statistically significant, the Spearman's correlation test would be conducted. The p-value was significant at $p \leq 0.05$.

RESULTS

Characteristics of the study subjects were presented in the table 1 below. The characteristics were the dependent (gender, age, duration of diabetes, BMI, waist circumference, waist-to-hip ratio, and laboratory test results of HbA1c, cholesterol, LDL, HDL and triglyceride) and independent (ultrasound-based NAFLD

grade) variable parameters shown in number, percentage and mean. In this current study, 78 subjects (95,1%) had NAFLD. Twenty-seven subjects (32,9%) had NAFLD grade 1 based on the ultrasound examination. Grade 2 and grade 3 of NAFLD were found in 40 (48,8%) and 11 (13,4%) subjects, respectively.

Table 1. The characteristics of study subjects

Parameter	Number, Percentage and Mean
Gender	
- Male	34 (37,8%)
- Female	51 (62,2%)
Age (years)	
- 30-39	4 (4,9%)
- 40-49	12 (14,9%)
- 50-59	25 (32,9%)
- 60-69	22 (28,1%)
- 70-79	6 (7,3%)
Duration of diabetes (years)	
- < 5	44 (55,9%)
- ≥ 5	41 (52,1%)
Body mass index	
- < 25 (ideal)	41 (52,6%)
- ≥ 25 (high)	41 (52,4%)
Waist circumference (cm)	
- Male < 90, female < 80 (ideal)	22 (28,1%)
- Male ≥ 90, female ≥ 80 (high)	62 (78,9%)
Waist-to-hip ratio	
- Male < 0,9, female < 0,85 (ideal)	11 (13,9%)
- Male ≥ 0,9, female ≥ 0,85 (high)	71 (89,6%)
HbA1c level (%)	
- < 7 (good glycemic control)	17 (21,7%)
- ≥ 7 (poor glycemic control)	65 (82,9%)
Cholesterol (mg/dl)	
- < 200 (normal)	51 (65,4%)
- ≥ 200 (high)	27 (34,6%)
LDL (mg/dl)	
- < 100 (normal)	41 (52,6%)
- ≥ 100 (high)	37 (47,4%)
HDL (mg/dl)	
- Male ≥ 40, female ≥ 50 (normal)	52 (66,7%)
- Male < 40, female < 50 (low)	26 (33,3%)
Triglyceride (mg/dl)	
- < 150 (normal)	48 (61,5%)
- ≥ 150 (high)	30 (38,5%)
NAFLD grade (ultrasonography)	
- Grade 0	4 (4,9%)
- Grade 1	27 (32,9%)
- Grade 2	40 (48,8%)
- Grade 3	11 (13,4%)

Non-modifiable risk factors (table 2) in the current research were gender, age, and duration of diabetes. There was no significant difference in ultrasound-based NAFLD grades between two gender groups in the study ($p = 0.954$). The significant difference was found across age groups ($p = 0.028$), but the correlation test result was not significant ($p = 0.691$). The comparative test result showed no difference between the two groups of diabetes duration ($p = 0.525$). Table 2. Ultrasound-based NAFLD grades across non-modifiable risk factor groups

	Ultrasound-based NAFLD grades			
	Grade 0	Grade 1	Grade 2	Grade 3
Gender (Mann-Whitney p-value = 0.954)				
Male	1	11	15	4
Female	2	16	25	7
Age/years (Kruskal-Wallis p-value = 0.028*, Spearman's rank p-value = 0.591)				
50-59	1	0	2	1
60-69	1	6	5	0
70-79	1	5	12	9
80-89	1	13	18	1
90-99	0	3	3	0
Duration of diabetes years (Mann-Whitney p-value = 0.523)				
< 5 years	2	12	21	6
≥ 5 years	2	15	19	5

*p-value ≤ 0.05

The anthropometric parameters (table 3) used to study the obesity risk factors were BMI, waist circumference and waist-to-hip ratio. The significant difference of comparative test was found between two BMI groups (p = 0.000) and the correlation test result was also significant (p = 0.000, r = 0.390). The p-value of comparative test between two waist circumference groups was 0.063 (no significant difference). There was no significant difference either between two waist-to-hip ratio groups (p = 0.371).

Table 3. Ultrasound-based NAFLD grades across anthropometric parameters (obesity risk factors)

	Ultrasound-based NAFLD grades			
	Grade 0	Grade 1	Grade 2	Grade 3
Body mass index (Mann-Whitney p-value = 0.000*, Spearman's rank p-value = 0.390*, r = 0.390)				
Low	2	19	18	1
High	1	8	22	10
Waist circumference (Mann-Whitney p-value = 0.063)				
Normal	1	10	8	1
High	3	17	32	10
Waist-to-hip ratio (Mann-Whitney p-value = 0.371)				
Normal	0	3	6	2
High	4	24	34	9

*p-value ≤ 0.05

Laboratory test parameters (table 4) were checked in the patients to analyse diabetes (glycemic control) and dyslipidemia risk factors. The significant difference was not found between two glycemic control groups (p = 0.437). For the dyslipidemia risk factors, the significant difference was not found between two

groups of LDL (p = 0.966), HDL (p = 0.575) and triglyceride (p = 0.060) levels. The ultrasound-based NAFLD grades were significantly different across two total cholesterol (normal and high) groups (p = 0.033). The total cholesterol was positively correlated with the NAFLD grades (p = 0.033, r = 0.237).

Table 4. Ultrasound-based NAFLD grades across laboratory parameter groups

	Ultrasound-based NAFLD grades			
	Grade 0	Grade 1	Grade 2	Grade 3
Glycemic control HbA1c (Mann-Whitney p-value = 0.437)				
Good	1	7	7	2
Poor	2	20	33	9
Cholesterol (Mann-Whitney p-value = 0.033*, Spearman's rank p-value = 0.033*, r = 0.237)				
Normal	3	20	24	4
High	1	17	16	7
LDL (Mann-Whitney p-value = 0.966)				
Normal	0	7	9	2
High	4	20	31	9
HDL (Mann-Whitney p-value = 0.575)				
Normal	2	18	27	5
Low	2	9	13	6
Triglyceride (Mann-Whitney p-value = 0.060)				
Normal	4	17	23	4
High	0	10	17	7

*p-value ≤ 0.05

DISCUSSION

In the present study 95,1% of the type-2 diabetes patients had NAFLD. Other hospital-based studies have shown the similar high prevalence of NAFLD in type-2 diabetes patients. Based on a Chinese systematic review of 24 studies 54.31-64.92% of type-2 diabetes patients have been diagnosed with NAFLD (21). Liver biopsy-proven NAFLD diagnosis has even been made in 87% of the type-2 patients in the Indian hospital (22). Data from the study in the hospital in Jakarta, Indonesia has given information that 45.2% of type-2 diabetes patients had NAFLD (14). This means that the present study prevalence has

surpassed the one from the previous study in Indonesia by a high percentage.

The current study showed that there was no association between gender and ultrasound-based NAFLD grade. This contradicts the theory which explains that free fatty acid (FFA) degradation rate is lower in men than women. As a result, more FFA accumulates in the liver of men (23). Previous studies have supported the theory. More men have NAFLD than women in a meta-analysis study based on epidemiologic research (13). Interesting results of gender as the risk factor come from a systematic review about NAFLD in Asia. The study has shown that before age 50, the number of men having NAFLD is twice as high as the women's number but when they are older, more women will have NAFLD (24).

There was a significant difference in the NAFLD grades based on the ultrasound examination across age groups but no significant correlation was found in the study. A molecular biology theory supports the relationship between ageing and NAFLD. An experiment in mice which was comparable to ageing humans has shown that the lack of nicotinamide adenine dinucleotide (NAD⁺) is associated with NAFLD. This is because NAD⁺ is an important nucleotide as a co-factor in the mitochondrial activity in the liver. Thus, the deficiency of this element results in the

accumulation of liver FFA. The genetically-engineered mouse model and ageing human with NAFLD in the experimental study had the deficiency (25). Moreover, ageing is an independent risk factor of NAFLD-related liver fibrosis (26).

Obesity is a major risk factor for both type 2 diabetes and NAFLD. Urbanisation in Asia which has changed the society from the agricultural one to the industrial one is increasing the obesity prevalence because of the rife unhealthy lifestyle. The high BMI (≥ 25) as an anthropometric measurement for obesity diagnosis in relation with NAFLD may be misleading as some Asian people (8-19%) with BMI < 25 known as having 'lean' NAFLD (2,27). In the present study the higher BMI is associated with the increase of ultrasound-based NAFLD grades (the p-value of Spearman's rank test was significant, 0.000 and $r = 0.390$).

The aforementioned 'lean' characteristic of NAFLD in Asian population suggests that other anthropometric measurements such as waist circumference and waist-to-hip ratio to detect abdominal obesity as a more accurate indicator of NAFLD (2,12). However, none of the two anthropometric measurement sets of groups (ideal and high) were associated with ultrasound-based grades of NAFLD. As an explanation to these results, the cut-off points of the two measurements as the indicator to foresee that someone has

the potential to develop NAFLD may differ from one population to another (17).

Given the notion that NAFLD is the hepatic feature of metabolic syndrome, this explains why type 2 diabetes is strongly associated with the metabolic liver disease. Moreover, insulin resistance as the cause of type 2 diabetes is one of the major factors in the pathogenesis of NAFLD (28). Another reason why diabetes is associated with some liver diseases is that the liver plays an important role to regulate glucose metabolism. In a case-control study conducted in an American hospital, patients with higher durations of diabetes had higher risks of developing hepatocellular carcinoma (29). A hospital-based study in Nigeria has shown that there was no significant difference of diabetes duration in different ultrasound-based NAFLD grades of type-2 diabetes patients (20). No significant difference has been found in the presence of NAFLD between patients who had type-2 diabetes ≥ 5 years and the counterpart in a study in Jakarta (14). The NAFLD grades based on ultrasonography were not different significantly in the two diabetes duration groups in our study.

Glycemic control is imperative in efforts to prevent complications in type-2 diabetes patients. The A1c type of hemoglobin can be used to evaluate if someone has a long exposure of hyperglycemia (18). However, there was no

significant difference in ultrasound-based NAFLD grades between patients with good and poor glycemic control. There has not been a significant difference either in glycemic control between different NAFLD grades of the Nigerian type-2 diabetes patients (20). The glycemic control index which is a result of glycated albumin divided by HbA1c may be a better biomarker to predict the progression of NAFLD based on the ultrasound examination as the study in Korea has shown (30).

The only component of the lipid profile associated with ultrasound-based NAFLD grades in this study was total cholesterol. Total cholesterol in the two groups (high and normal total cholesterol) was positively correlated with the grades. Regardless of the ethnicity background and locations, dyslipidemia is a major risk factor of NAFLD. Dyslipidemia prevalence along with obesity is increasing in Asia and strongly associated with the prevalence increase of NAFLD (31,32). A study proved that there is a significant link between dyslipidemia (the increase of total cholesterol, LDL, triglyceride, and the decrease of HDL) and the increase grades of NAFLD based on the ultrasound examination (19).

Several limitations might have occurred in this study. The sample number might not be enough to represent the total

number of type-2 diabetes patients who visit the hospital. The risk factor categories that had a big discrepancy of the patient number in the grouping appeared to have an insignificant statistical result. Another limitation might come from the diagnostic tool. The ultrasound device may not detect hepatic steatosis when it is lower than 20%. Its grading system can hardly be correlated with the histological severity categories. Fibrosis in NASH cannot be detected. The subcutaneous fat might also cause false echogenicity (15,16). As a solution, the scoring system that combines ultrasound-based grades with metabolic features might help (33).

CONCLUSION

The risk factors associated with NAFLD grades based on ultrasonography are age, BMI and total cholesterol. The higher BMI and total cholesterol are correlated with the increase of the NAFLD grades. These results suggest that the importance of lifestyle modification (healthy diet and exercise) to prevent NAFLD progression should be promoted among type-2 diabetes patients.

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