Relationship between ultrasound features of nonalcoholic fatty liver disease and cardiometabolic risk factors in patients with newly diagnosed type 2 diabetes

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INTRODUCTION It is suggested that nonalcoholic fatty liver disease (NAFLD) correlates with cardiometabolic risk factors in patients with newly diagnosed type 2 diabetes.

OBJECTIVES The aim of this study was to evaluate the prevalence of ultrasound features of NAFLD in patients with newly diagnosed type 2 diabetes and their relationship with cardiometabolic risk factors.

PATIENTS AND METHODS The study included 100 consecutive patients (mean age, 55.64 ± 13.42 years) with newly diagnosed type 2 diabetes, without other causes of hepatosteatosis. In each patient, medical history was taken, physical and abdominal ultrasound examinations were performed, and anthropometric and biochemical parameters were measured. Based on the results of an ultrasound examination, patients were assigned to 2 groups: with (n = 71) and without (n = 29) NAFLD.

RESULTS NAFLD was present in more than 70% of the patients with diabetes. In patients with NAFLD, significantly higher mean values of body weight, waist and hip circumferences, body mass index, liver enzyme activity, serum C-reactive protein, total cholesterol, and triglycerides and significantly lower levels of high-density lipoprotein (HDL) cholesterol were observed. There were no significant differences in the parameters of glycemic control between the groups. A correlation was observed between ultrasound features of NAFLD and some cardiovascular risk factors. Increased waist circumference and serum γ-glutamyltransferase level and decreased HDL-cholesterol levels were shown to be independent risk factors of NAFLD.

CONCLUSIONS Liver ultrasound should be performed in every patient with newly diagnosed type 2 diabetes. Our findings indicate a relationship between NAFLD and multiple cardiometabolic risk factors. The measurement of selected biochemical and anthropometric parameters may be used to assess the risk of NAFLD in this patient group.

KEY WORDS Cardiovascular risk factors, nonalcoholic fatty liver disease, type 2 diabetes mellitus, ultrasound examination
NAFLD is liver biopsy with histological assessment. However, this method has many limitations and is not useful in everyday clinical practice. An ultrasound examination of the liver has relatively high sensitivity (60%–95%) and specificity (88%–95%). Therefore, the diagnosis of NAFLD has been based so far on liver ultrasound examination and the measurement of laboratory parameters indicating liver injury or damage. Combining the results into various scores may strengthen the diagnosis. However, researchers are still looking for simple diagnostic tools with greater sensitivity and specificity that could serve as a screening test for excessive fat accumulation in the liver.

Growing evidence indicates the complex relation between diabetes mellitus and NAFLD with each condition being a prognostic factor for the other. A number of studies suggested that, in patients with type 2 diabetes, the dynamic progression of pathological changes in the liver may lead to cirrhosis. Additionally, it has been reported that NAFLD is associated with an increased risk of cardiovascular disease (CVD).

The main objective of this study was to estimate the prevalence of NAFLD early in the course of type 2 diabetes, with the use of ultrasonographic examination of the liver as a primary diagnostic tool. Additional objectives were to analyze the relationship between the results of liver ultrasound examination in diabetic patients with and without NAFLD with anthropometric measures, systolic and diastolic blood pressure, the results of selected biochemical parameters routinely assessed in diabetic population, as well as the presence of retinopathy.

**PATIENTS AND METHODS** The study involved 100 consecutive patients with newly diagnosed type 2 diabetes (34 women and 66 men), aged between 24 and 91 years (mean age, 55.64 ±13.42 years). All patients were hospitalized for hyperglycemia in the Department of Internal Disease, Diabetology and Clinical Pharmacology of the Medical University of Lodz, Łódź, Poland, between January 2006 and December 2009. In each patient, diabetes was diagnosed for the first time just before or during hospitalization based on the World Health Organization criteria.

Detailed medical history concerning clinical symptoms of type 2 diabetes and its occurrence in family members was taken on admission. Patients with other potential causes of hepatic steatosis (Table 1), including those consuming more than 10 g of alcohol per day, were excluded from the study. A physical examination was performed and anthropometric parameters (body mass, height, waist and hip circumference) and blood pressure (the intermediate value of 3 measurements) were assessed. Based on the obtained results, the body mass index (BMI) and waist-to-hip ratio (WHR) were calculated.

Fasting blood samples were taken to determine routine laboratory parameters: erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (ASP), γ-glutamyltransferase (GGT), total bilirubin, total protein, urea, creatinine, glucose, hemoglobin A1c, insulin, uric acid, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides (TG). The estimated glomerular filtration rate and the homeostasis model of assessment – insulin resistance (HOMA-IR) were calculated. Additionally, concentration of hepatitis B surface antigen and anti-HVC antibodies were measured to assess hepatotropic viral infections. Urine samples were taken to determine proteinuria, and dilated fundus examination was performed to assess the presence of diabetic retinopathy.

Abdominal ultrasonography was performed with particular emphasis on excessive hepatic steatosis. This examination was conducted by an experienced radiology specialist using Medison ACCUVIX V10 (Samsung Medison, Korea) according to the standard criteria. Based on ultrasound findings, patients were assigned to 2 groups: with (n = 71) and without (n = 29) NAFLD.

The study was approved by the Ethics Committee of the Medical University of Lodz. Each participant gave written informed consent prior to enrollment.

### Table 1: Potential causes of hepatic steatosis

| Category                  | Causes                                                                 |
|---------------------------|------------------------------------------------------------------------|
| Environmental hepatotoxins| Amanita phalloides mushrooms, phosphorous poisoning, petrochemicals, and Bacillus cereus |
| Inborn errors of metabolism| Abetalipoproteinemia, familial hypobetalipoproteinemia, familial combined hyperlipidemia, glycogen storage disease, Weber–Christian disease, lipodystrophy (congenital), familial hepatosteatosis, galactosemia, hereditary fructose intolerance, homocystinuria, systemic carnitine deficiency, tyrosinemia, Refsum’s syndrome, Schwachman syndrome, Wilson disease |
| Infections                | Hepatitis C virus (genotype 3), human immunodeficiency virus |
| Nutritional causes        | Total parenteral nutrition, severe surgical weight loss, starvation, refeeding syndrome |
| Other causes              | Celiac disease, Wilson’s disease, jejunal diverticulitis with bacterial overgrowth, inflammatory bowel disease, gastric bypass, jejunoileal bypass |
features of NAFLD as well as the optimal combination of sensitivity and specificity. The SPSS 12.00 package (IBM Corporation, United States) was used to perform all statistical analyses.

**Results**

Of 100 patients with newly diagnosed type 2 diabetes, 71 presented with ultrasound features of NAFLD (48 men and 23 women; mean age, 56.37 ±13.51 years), while 29 patients did not show features of hepatic steatosis (18 men and 11 women; mean age, 53.86 ±13.77). Comparative characteristics of the patients with and without ultrasonographic features of NAFLD are described in tables below.

### Table 2: Baseline anthropometric parameters in type 2 diabetic patients with and without ultrasound features of nonalcoholic fatty liver disease

| Variable                  | With NAFLD (n = 71)   | Without NAFLD (n = 29) | P value |
|---------------------------|-----------------------|-------------------------|---------|
| age, y                    | 56.37 ± 13.51         | 53.86 ± 13.77           | 0.40    |
| height, cm                | 169.18 ± 9.68         | 166.90 ± 10.10          | 0.29    |
| body mass, kg             | 89.33 ± 19.04         | 80.01 ± 18.13           | 0.02    |
| BMI, kg/m²                | 31.10 ± 5.62          | 28.79 ± 6.47            | 0.02    |
| waist circumference, cm   | 107.10 ± 12.35        | 96.83 ± 14.47           | <0.01   |
| hip circumference, cm     | 109.72 ± 30.36        | 103.24 ± 15.58          | <0.01   |
| WHR                       | 0.98 ± 0.08           | 0.94 ± 0.08             | 0.03    |
| systolic blood pressure, mmHg | 136.76 ± 23.33       | 144.14 ± 29.28          | 0.24    |
| diastolic blood pressure, mmHg | 83.38 ± 12.98        | 84.66 ± 13.49           | 0.74    |

Results are presented as mean ± standard deviation (range).

### Table 3: Baseline laboratory parameters in type 2 diabetic patients with and without ultrasound features of nonalcoholic fatty liver disease

| Variable                  | With NAFLD (n = 71)   | Without NAFLD (n = 29) | P value |
|---------------------------|-----------------------|-------------------------|---------|
| ESR, mm/h                 | 21.82 ± 18.83         | 17.66 ± 17.78           | 0.06    |
| CRP, mg/dl                | 0.89 ± 1.07           | 0.47 ± 0.45             | <0.01   |
| ALT, U/l                  | 46.59 ± 51.87         | 29.14 ± 22.81           | 0.01    |
| AST, U/l                  | 33.65 ± 31.48         | 21.76 ± 14.57           | <0.01   |
| GTP, U/l                  | 89.45 ± 79.37         | 45.33 ± 25.01           | <0.01   |
| total bilirubin, µmol/l   | 12.58 ± 7.87          | 10.36 ± 4.15            | 0.37    |
| total protein, g/dl       | 6.78 ± 0.91           | 6.73 ± 0.83             | 0.61    |
| urea, mmol/l              | 6.39 ± 4.85           | 6.41 ± 3.59             | 0.94    |
| creatinine, µmol/l        | 78.14 ± 32.27         | 79.03 ± 43.88           | 0.74    |
| GFR, ml/min/1.73 m²       | 127.52 ± 55.63        | 119.92 ± 52.72          | 0.53    |
| proteinuria, mg/dl        | 40.16 ± 32.54         | 32.01 ± 12.25           | 0.81    |
| glucose, mmol/l           | 16.05 ± 8.78          | 16.35 ± 8.72            | 0.95    |
| HbA₁c, %                  | 9.45 ± 2.50           | 9.92 ± 2.70             | 0.41    |
| insulin, µIU/ml           | 11.37 ± 5.17          | 10.13 ± 5.54            | 0.29    |
| HOMA-IR                   | 8.02 ± 6.19           | 7.47 ± 6.18             | 0.48    |
| uric acid, µmol/l         | 342.82 ± 143.32       | 302.79 ± 98.03          | 0.15    |
| TC, mmol/l                | 5.49 ± 2.25           | 4.77 ± 2.31             | <0.01   |
| LDL-C, mmol/l             | 3.13 ± 1.18           | 2.76 ± 0.91             | 0.13    |
| HDL-C, mmol/l             | 1.08 ± 0.52           | 1.20 ± 0.24             | <0.01   |
| TG, mmol/l                | 3.35 ± 3.70           | 2.90 ± 4.87             | <0.01   |

Results are presented as mean ± standard deviation.

### Statistical analysis

Demographic parameters and results of laboratory tests were summarized by their mean (standard deviation). The normality of the distribution was assessed with the Shapiro–Wilk’s W test. Comparisons of means between the groups were tested using the nonparametric Mann–Whitney U test, t test, and the independence χ² test. A P value of less than 0.05 was used to indicate statistical significance. A univariate correlation was assessed with stepwise logistic regression. Receiver operating characteristic curves were used to calculate clinically relevant cut-off values for variables independently associated with the presence of ultrasonographic features of NAFLD as well as the optimal combination of sensitivity and specificity.

The SPSS 12.00 package (IBM Corporation, United States) was used to perform all statistical analyses.

### RESULTS

Of 100 patients with newly diagnosed type 2 diabetes, 71 presented with ultrasound features of NAFLD (48 men and 23 women; mean age, 56.37 ±13.51 years), while 29 patients did not show features of hepatic steatosis (18 men and 11 women; mean age, 53.86 ±13.77). Comparative characteristics of the patients with and without ultrasonographic features of NAFLD are presented in tables above.
shown in Tables 2 and 3. Body mass, waist and hip circumferences, BMI, and WHR were significant-
ly higher in patients with NAFLD. There were no statistical differences in the frequency of dia-
betic retinopathy (9.86% vs. 13.79, \( P = 0.1977 \)) and family history of type 2 diabetes (70.42% vs.
68.97%, \( P = 0.8853 \)) between both groups. Additionally, in type 2 diabetic patients with NAFLD,
liver enzyme activity, TC, TG, and CRP concen-
trations were higher, while the mean HDL choles-
terol concentration was lower. Markers of meta-
bolic control, renal function, and ESR did not dif-
fer significantly.

The proposed cut-off points for cardiometabo-
linc risk parameters of NAFLD diagnosed by ultrasound examination and their odds ratio
(OR), sensitivity, and specificity are shown in Table 4. The highest predictive value for NAFLD
was shown for HDL cholesterol concentration of 1.05 mmol/l and lower, increasing this risk ap-
proximately 5-fold. A BMI of 2.7.5 kg/m² and higher (OR, 4.87; 95% confidence interval [CI],
2.00–11.87), waist circumference of 101.5 cm and higher (OR, 5.25; 95% CI, 1.86–11.00), and hip circumference of 103.5 cm and higher (OR, 4.17; 95% CI, 1.72–10.10) also had high predictive val-
ues for the development of NAFLD. The lowest value was demonstrated for WHR of 0.95 and
higher (OR, 2.13; 95% CI, 0.89–5.08); however, at this level, the risk of NAFLD increased more
than twice. The highest diagnostic sensitivity for NAFLD was shown for a BMI of 2.79 kg/m² and higher (70%) and the highest specificity for TC of 4.75 mmol/1 and higher, HDL cholesterol of
1.105 mmol/l and lower, and TG of 1.88 mmol/l and higher (about 69%). Stepwise logistic regres-
sion revealed that an increase in GTP levels as well as waist circumference were independently associated with an increased risk of NAFLD. The risk of NAFLD also increased with decreasing HDL cholesterol concentrations (Table 4).

**DISCUSSION** To the best of our knowledge, our study has been the first in the Polish population
to assess the frequency of NAFLD in newly di-
agnosed type 2 diabetes and correlate its pres-
ence with known and emerging cardiometab-
olic risk factors. We revealed that ultrasonog-
graphic features of NAFLD were present in more than 70% of the study participants as early as at di-
agnosis of diabetes. These results are consistent with the observation of other authors in different populations. It is reported that the incidence of NAFLD in obese patients and patients with type 2 diabetes is high and ranges from 70% to near-100%. It is estimated that NAFLD may affect about 90% of obese subjects in Poland, placing them at an increased risk of hepatitis and/or he-
patitic cirrhosis and possibly also CVD.

It is regarded that the presence of ultrasonog-
graphic features of NAFLD is an independent risk factor for CVD such as coronary heart disease, hy-
pertension, heart failure, and stroke. Hama-
guchi et al. showed that the incidence of acute cardiovascu-
lar events was higher in patients with ultrasound features of NAFLD. The results of nu-
merous studies suggest that fatty liver increas-
es an association of metabolic syndrome and/or type 2 diabetes with subclinical atheroscle-
rosis. Of note, this relationship was independent from conventional risk factors for CVD. Re-
cently, the occurrence of NAFLD in obese individuals has been highlighted. It was found that the presence of NAFLD in these subjects increases the risk of serious cardiovascular events. Indeed, as in our study, the study subpopulation of patients with NAFLD was characterized by significantly high-
er body weight, BMI and WHR, and greater waist
and hip circumferences. The results of our study demonstrated that waist circumference was an in-
dependent risk factor for ultrasonographic fea-
tures of NAFLD. Additionally, it was calculated that an increase in waist circumference by 1 cm increased the risk of NAFLD by 6.3%.

The results of several recent studies have shown the relationship between ultrasound features of steatosis and elevated transaminase levels in pa-
ients with long-standing diabetes. Our findings
confirm these observations; however, it is important to remember that we observed elevated liver enzymes in patients with NAFLD as early as at diagnosis of type 2 diabetes. Therefore, our results are in agreement with the recent findings of Zhang et al. suggesting the clinical usefulness of determining the activity of ALT and AST in estimating the risk of diabetes. It was further suggested that determination of ALT may be useful in estimating the risk of cardiovascular and cardiometabolic death. It is also regarded that another liver enzyme, GTP, may be a new risk factor for cardiometabolic diseases, since the relationship between elevated GTP levels and the risk of morbidity and mortality from CVD was observed. Our results indicate that an increase in GTP activity by 1 U/l increases the risk of NAFLD by 2.7%. Therefore, this observation supports the suggestion of other authors to determine the GTP level in the diagnosis of NAFLD.

The usefulness of other biochemical markers of liver function, such as bilirubin, in the diagnosis of NAFLD has not been confirmed. Similarly, the results of the study by Neuman et al. did not confirm the usefulness of ESR in the diagnosis of NAFLD, and the available data provided conflicting findings about the relationship of CRP with ultrasound features of NAFLD.

Particular attention in the diagnosis of NAFLD is paid to lipid disorders. The results of our study also confirmed this association. Patients with NAFLD had significantly higher concentrations of TC and TG and lower of HDL cholesterol. However, there were no differences in LDL cholesterol levels between the compared groups. These observations are consistent with the previous findings of Targhera et al. Epidemiological studies indicated a close relationship of low HDL cholesterol and the risk of major vascular events. It is known that low HDL cholesterol presents atherogenic phenotype and is associated with insulin resistance, obesity, disorders of carbohydrate metabolism, and hypertension. Our results showed that among the various components of lipid fractions, only low HDL cholesterol values were independently associated with an increased risk of NAFLD. We also demonstrated that an increase in HDL cholesterol concentration of 1 mmol/l increases the risk of NAFLD by 86.1%.

Several studies have suggested that insulin resistance and an increase in the percentage of glycated hemoglobin in patients with NAFLD are associated with prediabetes and newly diagnosed type 2 diabetes. We did not observe a similar association in our study because there were no significant differences between the groups of patients with and without NAFLD with respect to insulin, fasting glucose, and HOMA-IR. Lee et al. showed that the presence of ultrasonographic features of NAFLD correlated with elevated levels of uric acid; however, as in the case of the parameters of glucose metabolism, we did not observe a similar association in our population.

Our study has several limitations. Most importantly, the study group was relatively small. It would be important to confirm these results in a larger cohort. However, study participants belonged to one ethnic group, which, in our opinion, strengthens the results. On the other hand, we cannot be sure that predictive and cut-off values apply also to other populations. It is also important to stress that ultrasonography was the only method of diagnosis of NAFLD, and it was not confirmed by liver biopsy. However, none of the study participants agreed to liver biopsy.

In conclusion, liver ultrasound examination should be considered in every patient with newly diagnosed type 2 diabetes because the prevalence of NAFLD is extremely high in this group of patients. Our findings indicate a two-way relationship between NAFLD and multiple cardiometabolic risk factors. It seems that specific threshold values for several biochemical and anthropometric measurements may be used to assess the risk of NAFLD in diabetic population.

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Związek między ultrasonograficznymi cechami niealkoholowego tłuszczenia wątroby a czynnikami ryzyka kardiometabolicznego u pacjentów ze świeżo zdiagnozowaną cukrzycą typu 2

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STRÓŻCZENIE

WPROWADZENIE Sugeruje się, że obecność ultrasonograficznych cech niealkoholowego tłuszczenia wątroby (nonalcoholic fatty liver disease – NAFLD) koreluje z czynnikami ryzyka kardiometabolicznego u pacjentów ze świeżo rozpoznaną cukrzycą typu 2.

CELE Celem badania była ocena częstości występowania ultrasonograficznych cech NAFLD u pacjentów ze świeżo rozpoznaną cukrzycą typu 2 oraz ich związek z czynnikami ryzyka kardiometabolicznego.

PACjENTI I METODY Do badania włączono 100 kolejnych pacjentów (średnia wieku 55,64 ± 13,42 roku) ze świeżo rozpoznaną cukrzycą typu 2, u których wykluczono inne przyczyny stłuszczenia wątroby. U każdego pacjenta przeprowadzono badanie podmiotowe, przedmiotowe, wykonano badanie ultrasonograficzne jamy brzusznej oraz oceniono parametry antropometryczne i biochemiczne. Pacjentów przydzielono do dwóch grup na podstawie wyniku badania ultrasonograficznego – z (n = 71) i bez (n = 29) NAFLD.

WYNIKI Obecność NAFLD stwierdzono u >70% pacjentów z cukrzycą. W grupie chorych z NAFLD stwierdzono istotnie wyższe średnie wartości masy ciała, obwodu pasa i bioder, wskaźnika masy ciała (body mass index – BMI), aktywności enzymów wątrobowych, stężenia białka C-reaktywnego, cholesterolu całkowitego, triglicerydów oraz niższe stężenie cholesterolu HDL. Nie wykazano znamiennej różnicy między porównywanymi grupami pacjentów we wskaźnikach kontrol gospodarki węglowodanowej. Dodatkowo stwierdzono korelację między ultrasonograficznymi cechami NAFLD a niektórymi czynnikami ryzyka sercowo-naczyniowego. Wykazano, że zwiększenie obwodu w pasie, poziomu gamma-glutamylotransferazy i zmniejszenie stężenia cholesterolu HDL są niezależnymi czynnikami ryzyka wystąpienia NAFLD.

WNIOSKI Badanie ultrasonograficzne wątroby powinno być wykonane u każdego pacjenta ze świeżo zdiagnozowaną cukrzycą typu 2. Uzyskane wyniki wskazują na związek między NAFLD a wieloma czynnikami ryzyka kardiometabolicznego. Ocena wybranych parametrów biochemicznych i antropometrycznych może być przydatna w szacowaniu ryzyka wystąpienia NAFLD w tej grupie chorych.