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Clinical spectrum of non-alcoholic fatty liver disease in diabetic and non-diabetic patients ☆

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ABSTRACT

Background: While non-alcoholic fatty liver disease (NAFLD) has been well characterised in patients with diabetes mellitus (DM), less is known about NAFLD in non-DM patients. We investigated the clinical characteristics of NAFLD patients with and without DM and accuracy of the NAFLD fibrosis score (NFS) in these two NAFLD groups.
Methods: Clinical, biochemical and histological variables were evaluated in this prospective cross-sectional study of 503 patients with biopsy proven NAFLD. Comparisons between patients with and without DM were analysed. NFS was correlated with liver histology to assess its robustness in patients with and without DM.
Results: There were 503 biopsy proven NAFLD patients with 48% of the cohort being diabetic. Relative to patients without DM, patients with DM were older (52 vs. 46 years, $p < 0.001$), with higher proportion of females (70% vs. 54%, $p < 0.001$), higher BMI (37 vs. 35, $p = 0.009$), higher prevalence of hypertension (73% vs. 44%, $p < 0.001$), higher prevalence of NASH (80.2% vs. 64.4%; $p < 0.001$) and advanced fibrosis (40.3% vs. 17.0%; $p < 0.001$). A considerable amount of patients without DM still had NASH (64%) and advanced fibrosis (17%). The clinical utility of the NFS differed between NAFLD patients with and without DM, with sensitivity to exclude advanced fibrosis being 90% of NAFLD patients with DM but only 58% of patients without DM.
Conclusion: Patients with DM have more severe NAFLD based on histology. However, NASH and advanced fibrosis also occur in a considerable proportion of NAFLD patients without DM. The lower utility of the NFS in NAFLD patients without DM emphasises the heterogeneous nature of the NAFLD phenotype.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of metabolic syndrome [1], represents a spectrum of histopathologic abnormalities ranging from simple steatosis to the more aggressive non-alcoholic steatohepatitis (NASH), characterised by steatosis, parenchymal inflammation, hepatocellular ballooning and other evidence of hepatic injury [2]. Patients with NASH are at risk of developing progressive fibrosis; reported in up to 50% of cases over 6 years [3]. There is increasing recognition that NAFLD is a heterogeneous disease with multiple pathways of pathogenesis and patients with different phenotypes of NAFLD can present with diverse disease manifestations [4]. Insulin resistance plays a dominant role in the pathogenesis of NAFLD [5]. Patients with type 2 diabetes mellitus (DM) have an increased risk of developing NAFLD, NASH and hepatic fibrosis/cirrhosis [6–9]. Furthermore, NAFLD patients with DM have three times the mortality compared to non-

Abbreviations: ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ACE-I, angiotensin-converting enzyme-inhibitor; ARB, angiotensin receptor blocker; apoB-100, apolipoprotein B-100; BMI, body mass index; CIs, confidence intervals; Chol, total cholesterol; DM, type 2 diabetes mellitus; ER, endoplasmic reticulum; FFAs, free-fatty acids; HDL, high density lipoprotein cholesterol; HOMA-IR, Homeostatic model assessment—insulin resistance; INR, international normalised ratio; LDL, low density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NFS, NAFLD fibrosis score; NASH CRN, Non-alcoholic Steatohepatitis Clinical Research Network; NAS, NAFLD activity score; ORs, odd ratios; SDs, standard deviations; TGs, triglycerides; VLDL, very-low-density lipoproteins

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diabetic NAFLD patients [10]. The importance of DM in NAFLD is reflected by its inclusion in the majority of the non-invasive composite predictive scores for NASH and advanced fibrosis [11–14]. One such composite predictive score for predicting advanced fibrosis in NAFLD is the NAFLD fibrosis score (NFS), which has been validated and recommended for use in the American society guidelines [2,14]. Reiterating disease heterogeneity and that NAFLD may not conform to a "one size fits all approach", McPherson and colleagues had reported a difference in the reliability of NFS in the context of normal and abnormal ALT levels [15]. Other non-invasive fibrosis scores such as the BARD score and AST/ALT ratio have also been used to predict advanced fibrosis in NAFLD. We sought to characterise the clinical spectrum of NAFLD in patients with and without DM. In addition, we explored the utility of NFS and other established non-invasive fibrosis scores among these two groups.

2. Materials and methods

2.1. Study design and population

This is a prospective cross sectional study with patients enrolled from two hepatology outpatient clinics in Cleveland, Ohio (Cleveland Clinic and MetroHealth Medical Center). Study received approval from the institutional review board.

The study included patients 18 years of age and over, with histologically proven NAFLD, who had not received any prior therapies that may have been beneficial for NAFLD, such as Vitamin E, pentoxifylline, pioglitazone and prescribed diet & exercise weight loss programmes. Patients with excessive alcohol consumption (>21 drinks per week and >14 drinks per week for males and females respectively) were excluded. Similarly, patients with other contributory causes of liver disease including those with hepatotoxic drug history, viral hepatitis, hemochromatosis, autoimmune hepatitis, Wilson's disease or alpha 1 antitrypsin disease were excluded.

2.2. Ascertainment of clinical data

Demographic and clinical information was obtained by two of the authors (SD or AM) for all patients from an electronic medical record system that is common to both hospitals. The diagnosis of DM was diagnosed based on American Diabetes Association (ADA) criteria with or without the use of antidiabetic medications [16]. Hypertension was diagnosed by the Joint National Committee (JNC) 7 criteria [17]. All diagnoses were verified based on documentation in the electronic medical records by one of the investigators (SD or AM). Body mass index (BMI) were collated, as were liver function tests [serum albumin, bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT)], platelet count, international normalised ratio (INR), total cholesterol (Chol), triglycerides (TGs), high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and HbA1C. To minimise inter-observer variability, liver biopsy specimens were read using standardised well defined histological criteria [18] established by the Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN). As recommended, the diagnosis of NASH was based on the overall impression of histopathologists and not the NAFLD activity score [NAS] [19]. Fibrosis was classified into 4 stages with advanced fibrosis defined as stage 3–4 fibrosis (bridging fibrosis–cirrhosis). Only clinical variables obtained within 6 months of the liver biopsy were included in the analysis. The use of statins and angiotensin-converting enzyme-inhibitor (ACE-I)/angiotensin receptor blocker (ARB) within 6 months prior to the liver biopsy was also examined. Insulin resistance was assessed with Homeostatic model assessment—insulin resistance (HOMA-IR) based on the formula: [fasting glucose (mg/dL) × insulin (μU/mL)] divided by 405 [20]. NFS was calculated according to the published formula; NFS: $-1.675 + 0.037 \times$ age (years) $+ 0.094 \times$ body mass index (BMI; kg/m^2) $+ 1.13 \times$ impaired fasting glycaemia or DM (yes = 1, no = 0) $+ 0.99 \times$ AST/ALT ratio $- 0.013 \times$ platelet ($\times 10^9$/L) $- 0.66 \times$ albumin (g/dL) [14]. The cut-off points of the NFS used to categorise fibrosis were: <−1.455, −1.455 to 0.676, and >0.676 for low indeterminate and high probability for advanced fibrosis, respectively [14]. Calculation of the BARD score was also performed; the BARD score was a 4 point score derived from the weighted sum of three variables (BMI > 28 = one point, AST/ALT ratio > 0.8 = two points, diabetes = one point) where a score of two or more suggestive of advanced fibrosis [13]. Similarly, AST/ALT ratio more than 0.8 itself has been suggested to be useful in predicting advanced fibrosis [21].

2.3. Statistical analysis

Descriptive statistics was computed for all variables and reported as means and standard deviations (SDs) for continuous variables or frequencies and percentages for categorical variables. Baseline characteristics and differences in demographic, clinical, histological and laboratory indices between patients with and without DM were ascertained, using Student's T tests and Pearson's chi-square testing for continuous and categorical variables, respectively. Mann–Whitney U test was also performed when applicable. The utility of NFS was correlated with histological staging of fibrosis in both DM and non-DM patients. With regard to advanced fibrosis, Spearman's correlation analysis for each individual component of the NFS was performed in both patients with and without DM. In addition, the utility of using fibrosis scores such as the BARD score and AST/ALT ratio was also assessed in both patients with and without DM. All analyses were performed using SPSS version 21 statistical software (Chicago, Illinois, USA). Two sided p values were used. p values < 0.05 were considered statistically significant.

3. Results

Of the 503 NAFLD patients in the data set, 62% were female, 58% had concomitant hypertension, 48% had concomitant DM and the mean BMI was 36.1 kg/m^2. NASH and advanced fibrosis were present in 71.8% and 28.1% of the cohort respectively.

Table 1 illustrates the characteristics of the whole cohort and also patients with and without DM. Compared to NAFLD patients without DM, patients with DM were older (52.5 vs. 46.0 years; $p < 0.001$), were more likely to be female (70.7% vs. 54.0%; $p < 0.001$) and have a higher BMI (37.2 vs. 35.2 kg/m^2; $p = 0.009$). Diabetic patients were also more likely to be hypertensive (73.2 vs. 43.9%; $p < 0.001$) with greater use of statins (42.0 vs. 15.2%; $p < 0.001$) and ACE-I/ARBs (51.9 vs. 28.8%; $p < 0.001$). However, there was no difference in the prevalence of advanced fibrosis among the DM patients taking or not taking statins ($p = 0.182$) and also among the DM patients taking or not taking ACE-I/ARB ($p = 0.357$). There were differences in ALT, albumin, platelet count, TG, LDL and HbA1C between the two groups. There was no difference in aggregated length of liver tissue examined between patients with and without DM (19.9 mm vs. 18.2 mm; $p = 0.225$). As shown in Table 2, patients with diabetes had more lobular inflammation ($p = 0.017$), ballooning ($p < 0.001$) and NASH ($p < 0.001$). The median NAS was higher in DM patients compared to non-DM patients ($p = 0.022$). More of the DM patients had grade 2 ballooning (41.7% vs. 24.0%; $p < 0.001$) and higher prevalence of NASH (80.2% vs. 64.4%; $p < 0.001$) compared to non-DM patients, while there was no differences in steatosis between patients with and without DM. DM patients also had a higher prevalence of advanced fibrosis (40.3% vs. 17.0%; $p < 0.001$) [Table 1]. When cirrhosis was considered specifically, significantly more of the DM patients had cirrhosis relative to the non-DM patients (20.6% vs. 5.7%) [Table 2]. Among the DM patients, the duration of DM did not differ between those with and without advanced fibrosis (5.79 vs. 5.42 years, $p = 0.791$). Similarly, HbA1C levels were comparable between diabetic patients with and without advanced fibrosis (7.5 vs. 7.3, $p = 0.414$). Among the patients without DM, there was no