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Not only type 2 diabetes but also prediabetes is associated with portal inflammation and fibrosis in patients with non-alcoholic fatty liver disease

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ABSTRACT

Aims: Growing evidence suggests that not only type 2 diabetes (T2D) but also prediabetes (PD) is common in patients with non-alcoholic fatty liver disease (NAFLD). However, few data exist on how PD impacts the histological characteristics of NAFLD patients. In this exploratory study, we sought to investigate the associations of PD and T2D with the severity of the histological features in patients with NAFLD.
Methods: The population consisted of 280 patients with biopsy-proven NAFLD. The associations of PD and T2D with the severity of histological features of NAFLD were analyzed using multiple logistic (or ordinal logistic) regression models after adjustment for confounding factors.
Results: PD and T2D was noted in 102 (36.4%) and 92 (32.8%) of patients, respectively. Of the 92 patients with T2D, ten (10.9%) were diagnosed *de novo* after the OGTT. PD and T2D were significantly associated with more severe portal inflammation ($P < 0.01$); the adjusted odds ratios (ORs) of PD and T2D for having a higher grade of portal inflammation were 1.8 [95% CI, 1.1, 3.2] and 2.6 [95% CI, 1.3, 5.8]), respectively. A similar relationship was observed for liver fibrosis ($P < 0.001$); specifically, the adjusted ORs of PD and T2D for having a higher grade of hepatic fibrosis were 2.4 [95% CI, 1.3, 3.7] and 3.8 [95% CI, 1.9, 6.1]), respectively.
Conclusion: Not only T2D but also PD is independently associated with portal inflammation and fibrosis in NAFLD patients. PD may be useful as a clinical indicator of patients who are likely to have already more severe histological findings.

1. Introduction

Prediabetes (PD), which refers to an intermediate stage between normal glucose tolerance and overt type 2 diabetes (T2D), is defined by glycemic variables that are higher than normal, but lower than diabetes thresholds (Bergman, 2013; Centers for Disease Control and Prevention (CDC), 2013; Tabák, Herder, Rathmann, Brunner, & Kivimäki, 2012). Currently, PD may be diagnosed in the presence of at least one of the following criteria: 1) a fasting blood glucose level between 100 and 125 mg/dL; 2) a glycated hemoglobin between 5.7% and 6.4%; or 3) a blood glucose levels of 140–199 mg/dL after the oral glucose tolerance test (OGTT) (Mann et al., 2010). Previous studies have shown that 5%–10% of PD subjects per year will progress to T2D, with a similar proportion converting back to normal glucose tolerance (Nichols, Hillier, & Brown, 2007). The underlying pathophysiological disturbances of excess risk from prediabetes are believed to be the same as those from T2D, with a central role played by insulin resistance (Johnson, Duick, Chui, & Aldasouqi, 2010; Weiss et al., 2003).

Non-alcoholic fatty liver disease (NAFLD) is traditionally considered as the hepatic manifestation of insulin resistance and the metabolic syndrome (Oh, Winn, & Poordad, 2008; Vanni et al., 2010; Yilmaz, 2012). Moreover, the NAFLD is strongly associated in a complex and bidirectional manner with T2D (Anstee, Targher, & Day, 2013). Indeed not only has T2D the potential to promote the onset and progression of NAFLD, but also patients with NAFLD are at an increased risk of incident T2D (Anstee et al., 2013). Although substantial evidence supports the contention that PD is associated with endothelial dysfunction (Eringa et al., 2013), atherosclerotic vascular disease (Kramer, Raji, & Plutzky, 2003) and target-organ damage (Diamantopoulos et al., 2006), to date few data exist regarding the association between histologically-diagnosed NAFLD

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and PD. Ortiz-Lopez et al. (Ortiz-Lopez et al., 2012) have reported that newly diagnosed PD is considerably more common in patients with NAFLD than in those without NAFLD (75% vs. 25%). Moreover, a prospective cohort study by Zelber-Sagi et al. (Zelber-Sagi et al., 2013) demonstrated that ultrasound-diagnosed NAFLD is a strong and independent risk factor for PD in the general adult population. Preliminary data also suggested that PD states may have an impact on the histological characteristics of NAFLD patients. In 83 patients with biopsy-diagnosed NAFLD, Haukeland et al. (Haukeland et al., 2005) demonstrated that abnormal glucose tolerance (defined as either T2D or impaired glucose tolerance [IGT]) was an independent risk factor for non-alcoholic steatohepatitis (NASH) and fibrosis. In another small study conducted in 73 Chinese patients with biopsy-proven NAFLD, Wong and coworkers (Wong et al., 2006) reported that IGT was more common in patients with NASH than those with simple steatosis, and that 2-h plasma glucose levels after OGTT correlated with fibrosis stage.

In this multicenter retrospective exploratory study of prospectively collected data, we examined the associations of PD and T2D with the severity of histological features in a large sample of patients with NAFLD.

2. Materials and methods

2.1. Study patients

This is a retrospective review of a prospective database of patients with biopsy-proven NAFLD enrolled from four different gastroenterology clinics in Turkey. Liver biopsies were processed routinely, and scored by a single pathologist in each participating institution. The final study population consisted of 280 Turkish patients with NAFLD (147 males and 133 females; mean age, 45.6 ± 9.9 years). To be included in this study, patients were required to have a daily alcohol intake not exceeding 20 g/day. All of the study participants showed ultrasonographic evidence of steatosis grade 1 or higher. The exclusion criteria were as follows: type 1 diabetes, presence of viral hepatitis, primary biliary cirrhosis, sclerosing cholangitis, biliary obstruction, hemochromatosis, Wilson's disease, autoimmune hepatitis, alpha-1 antitrypsin deficiency, previous abdominal surgery, impaired renal function or malignancies. We also excluded patients taking estrogens, amiodarone, steroids, and tamoxifen. The adopted procedures were in agreement with the Helsinki Declaration. The protocol was approved by the Institutional Review Board of the Marmara University School of Medicine. Individual patient consent was obtained for entry into the database. However, our Institutional Review Board waived the need for individual patient consent for this study. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

2.2. Liver histology

Ultrasonography-guided liver biopsies were performed under conscious sedation using a 16-gauge Hepafix needle. All biopsy specimens were placed in formalin solution for fixation and embedded in paraffin blocks. Serial sections (sectioned at 4 mm intervals) were stained with hematoxylin–eosin and Masson's trichrome. All liver biopsy specimens were at least 20 mm long and/or contained more than 11 complete portal tracts. An experienced pathologist blinded to clinical data scored the liver biopsies according to the NIDDK NASH Clinical Research Network scoring system (Kleiner et al., 2005). Steatosis was scored from 0 to 3 with a four-grade scoring system from S0 to S3: S0: no steatosis or less than 5%, S1: 5%–33%, S2: 33%–66%, and S3: > 66%. Lobular inflammation was graded as follows: stage 0, no foci; stage 1: < 2 foci per 200× field; stage 2: 2–4 foci per 200× field; stage 3: > 4 foci per 200× field. Fibrosis was staged as follows: stage 0: no fibrosis; stage 1: perisinusoidal or periportal fibrosis with three different patterns — 1A: mild, zone 3, perisinusoidal; 1B: moderate, zone 3, perisinusoidal fibrosis, and 1C: portal/periportal fibrosis; stage 2: perisinusoidal and portal/periportal fibrosis; stage 3: bridging fibrosis; and stage 4: cirrhosis. The histological non-alcoholic steatohepatitis (NASH) score was defined as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3) and ballooning (0–2), thus ranging from 0 to 8 (Kleiner et al., 2005). Cases with scores of 0–2 were considered as having simple steatosis; on the other hand, cases with scores of 5 or greater were diagnosed as definitive NASH. Cases with activity scores of 3 and 4 were considered as borderline NASH.

2.3. Diagnosis of diabetes, prediabetes, and normal glucose tolerance

Fasting and 2-h oral OGTT plasma glucose levels were measured in all participants. Based on the American Diabetes Association (ADA) guidelines, we defined impaired fasting glucose (IFG) as fasting plasma glucose (FPG) levels 100 mg/dL (5.6 mmol/L) to 125 mg/dL

Table 1
General characteristics of NAFLD patients with of normal glucose tolerance, PD, and T2D.

Characteristic	Normal glucose tolerance (n = 86)	PD (n = 102)	T2D (n = 92)	*P* value
Age, years	42 ± 10	45 ± 10	49 ± 9	<0.001
Sex (males/females)	57/29	52/50	38/54	<0.01
Body mass index, kg/m^2	30.2 ± 4.4	31.4 ± 5.5	31.8 ± 5.4	0.11
Systolic blood pressure, mmHg	123 ± 17	126 ± 15	132 ± 18	<0.01
Diastolic blood pressure, mmHg	80 ± 12	82 ± 10	82 ± 11	0.58
Active smokers (yes/no)	21/65	18/84	23/69	0.39
AST, U/L	43 (32–58)	42 (30–55)	44 (32–58)	0.56
ALT, U/L	65 (51–98)	70 (41–99)	62 (47–96)	0.78
Total cholesterol, mg/dL	204 ± 42	221 ± 45	216 ± 50	<0.05
LDL cholesterol, mg/dL	129 ± 35	141 ± 39	136 ± 38	0.09
HDL cholesterol, mg/dL	44 ± 11	46 ± 10	44 ± 10	0.51
Triglycerides, mg/dL	165 (112–222)	154 (116–231)	175 (120–257)	0.37
Plasma glucose, mg/dL	91 ± 10	98 ± 11	134 ± 41	<0.001
Glycated hemoglobin, %	5.2 ± 0.4	5.7 ± 0.5	6.9 ± 1.4	<0.001
HOMA-IR	3 (2–4)	4 (2–5)	4 (3–6)	<0.01
C-reactive protein, mg/dL	0.57 (0.10–3.10)	0.64 (0.19–2.76)	0.99 (0.39–3.44)	0.16
Metabolic syndrome (yes/no)	39/47	64/38	77/15	<0.001

Data are reported as mean ± SD, median and interquartile ranges, or counts. *P* values were calculated using by one-way analysis of variance (ANOVA), the Kruskal–Wallis test, or chi-square tests, as appropriate.