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Microalbuminuria in nondiabetic patients with nonalcoholic fatty liver disease: association with liver fibrosis

Yusuf Yilmaz[a,*], Yesim Ozen Alahdab[a], Oya Yonal[a], Ramazan Kurt[a], Alla Eldeen Kedrah[a], Cigdem Ataizi Celikel[b], Osman Ozdogan[a], Deniz Duman[a], Nese Imeryuz[a], Erol Avsar[a], Cem Kalayci[a]

[a]*Department of Gastroenterology, Marmara University School of Medicine, Altunizade, Istanbul 34662, Turkey*
[b]*Department of Pathology, Marmara University School of Medicine, Altunizade, Istanbul 34662, Turkey*

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Abstract

Recent evidence has suggested an association between microalbuminuria and ultrasound-diagnosed nonalcoholic fatty liver disease (NAFLD) in patients with diabetes and prediabetes. However, few data are available on the occurrence of microalbuminuria in nondiabetic subjects with histologically proven NAFLD. We thus evaluated the relationships between microalbuminuria and liver histology in a hospital-based sample of 87 adults with biopsy-proven NAFLD from Turkey. An albumin excretion rate less than 30 mg/d was considered within the reference range, whereas an albumin excretion rate from 30 to 300 mg/d was considered to indicate microalbuminuria. Compared with those without microalbuminuria (n = 73), NAFLD patients with microalbuminuria (n = 14) had significantly higher homeostasis model assessment of insulin resistance values (3.9 ± 1.3 vs 5.8 ± 3.7, $P < .001$). There were no differences in the prevalence of microalbuminuria in patients with definite nonalcoholic steatohepatitis, borderline nonalcoholic steatohepatitis, and simple fatty liver. In the entire study cohort, mean fibrosis scores were significantly higher in patients with microalbuminuria than in those without (1.27 ± 0.26 vs 0. 80 ± 0.11, $P < .05$). This difference persisted after adjustment for potential confounders. These results indicate the presence of a significant association between the severity of insulin resistance and microalbuminuria in patients with NAFLD. In addition, microalbuminuria may identify NAFLD patients with higher fibrosis scores.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) represents the most frequently diagnosed cause of chronic liver disease in Western countries and includes a wide spectrum of hepatic injuries, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) [1]. Nonalcoholic fatty liver disease is increasingly recognized as the hepatic manifestation of the metabolic syndrome (MetS), and the MetS and all its clinical traits are highly prevalent in patients with NAFLD [2,3]. An association between microalbuminuria and the components of MetS has been described in many populations [4], and microalbuminuria is a clinical criterion for the MetS by the World Health Organization classification [5]. However, some studies have not confirmed this association [6]; and the inclusion of microalbuminuria in the MetS has been debated [7].

Preliminary data have suggested an association between microalbuminuria and NAFLD in patients with diabetes and prediabetes. Hwang and coworkers [8] have recently reported a strong independent relationship between microalbuminuria and ultrasound-diagnosed NAFLD in patients with prediabetes and newly diagnosed diabetes in a population-based study of 1361 Korean subjects. Interestingly, there is also evidence to suggest that NAFLD might increase the development or progression of microalbuminuria [9]. In pediatric NAFLD, however, Manco and colleagues [10] failed to demonstrate an association between urinary albumin excretion and insulin resistance.

* Corresponding author. Tel.: +90 5334403995; fax: +90 2166886681.
E-mail address: yusufyilmaz@uludag.edu.tr (Y. Yilmaz).

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The exact significance and correlates of microalbuminuria in adult nondiabetic subjects with biopsy-proven NAFLD remain to be determined. In addition, the diagnosis of NAFLD in previous studies among adult patients was based on ultrasound, but was not confirmed by liver biopsy, which is the best diagnostic tool for confirming NAFLD [11]. Therefore, the main aim of the present hospital-based, cross-sectional study was to examine whether there were significant associations between the severity of liver histopathology and the presence of microalbuminuria among NAFLD patients. We also assessed the clinical differences of NAFLD patients with and without microalbuminuria.

2. Subjects and methods

2.1. Study participants

Eighty-seven consecutive patients (48 men and 39 women; mean age, 47.0 ± 8.7 years; age range, 26-70 years) with NAFLD were recruited from the Department of Gastroenterology, Marmara University School of Medicine, Istanbul, Turkey. Potential participants with known diabetes were excluded from this study. An oral glucose tolerance test using a 75-g glucose load was applied to exclude diabetes in all subjects. Patients with viral hepatitis, hemochromatosis, Wilson disease, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, biliary obstruction, α-1 antitrypsin deficiency, ischemic cardiac or cerebrovascular disease, impaired renal function, daily alcohol intake exceeding 20 g/d, previous abdominal surgery, and malignancies were carefully excluded from the present study. Because false-positive results for microalbuminuria can occur in individuals with recent strenuous activity, urinary tract infections, or febrile illnesses, as well as in women who are pregnant or menstruating, these subjects were not included in this study. None of the subjects was using any medications, including estrogens, amiodarone, steroids, tamoxifen, or herbal supplements. Written informed consent was obtained from all participants. The study protocol was approved by the local Institutional Review Board.

2.2. Assessment of microalbuminuria

Twenty-four-hour urine samples were collected in a standardized manner, and all patients were advised of the collection method via a written instruction sheet. Measurement of 24-hour urinary albumin was performed in the central hospital laboratory using a BN Prospec (Dade Behring, Marburg, Germany) nephelometric system. An albumin excretion rate (AER) less than 30 mg/d was considered within the reference range, whereas an AER from 30 to 300 mg/d was considered to indicate microalbuminuria.

2.3. Clinical examinations

All subjects underwent physical examination, anthropometric measurements, and biochemical screening. Study participants underwent liver ultrasound scanning to assess the degree of steatosis. Liver steatosis was assessed semiquantitatively on a scale of 0 to 3: 0, absent; 1, mild; 2, moderate; and 3, severe. An experienced pathologist examined liver histology according to the National Institute of Diabetes and Digestive and Kidney Diseases NASH Clinical Research Network scoring system [12]. Patients with suspected NAFLD were classified according to their liver histology as definitive NASH, borderline NASH, or simple fatty liver.

2.4. Histologic analysis

Ultrasonography-guided liver biopsies were performed under conscious sedation using a 16-gauge Hepafix needle (Hepafix, Braun Melsungen AG, Melsungen, Germany). The length of histologic specimens was not smaller than 2.5 cm. 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, Masson trichrome. An experienced pathologist blinded to clinical data scored the liver biopsies according to the National Institute of Diabetes and Digestive and Kidney Diseases NASH Clinical Research Network scoring system [12]. Steatosis was scored from 0 to 3 with a 4-grades scoring system from S0 to S3: S0, no steatosis or less than 5%; S1, 5% to 33%; S2, 33% to 66%; S3, greater than 66%. Lobular inflammation was graded as follows: stage 0, no foci; stage 1: less than 2 foci per 200× field; stage 2, 2 to 4 foci per 200× field; stage 3, more than 4 foci per 200× field. Fibrosis was staged as follows: stage 0, no fibrosis; stage 1, perisinusoidal or periportal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, bridging fibrosis; stage 4, cirrhosis. The *histologic 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. Cases with scores of 0 to 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 (probable) NASH.

2.5. Data analysis

The study power was calculated using the StatMate software version 2.0 (GraphPad, San Diego, CA) for Windows. Our experiment had a 95% power to detect a difference between mean fibrosis scores of 0.26 with a significance level (α) of 0.05 (2-tailed). Data are given as the mean ± standard deviation or counts. Statistical analyses included an independent-sample t test and the χ^2 test (for categorical variables). Correlations were calculated using Pearson correlation coefficient. Analysis of covariance was used to standardize for differences in fibrosis scores across groups after allowance for possible confounders including age, sex, and homeostasis model assessment of insulin resistance (HOMA-IR) values. Two-tailed P values

less than .05 were considered statistically significant. All analyses were run using SPSS software version 17.0 (SPSS, Chicago, IL) for Windows.

3. Results

Overall, 73 patients (83.9%) had an AER less than the microalbuminuria cutoff point of 30 mg/d; and 14 (16.1%) had microalbuminuria. The general characteristics of NAFLD patients according to the presence or absence of microalbuminuria are shown in Table 1. Compared with those without microalbuminuria (n = 73), NAFLD patients with microalbuminuria (n = 14) had significantly higher HOMA-IR values (3.9 ± 1.3 vs 5.8 ± 3.7, $P < .001$). The association between microalbuminuria and HOMA-IR was similarly evident when AER was analyzed as a continuous variable ($r = 0.357$, $P < .001$). No significant intergroup differences were found with regard to age, sex, body mass index (BMI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), hypertension, triglycerides, high-density lipoprotein (HDL) cholesterol, and serum ferritin.

Liver histopathology of the NAFLD patients with microalbuminaria revealed steatosis alone in 1 subject, borderline NASH in 3 subjects, and definite NASH in 10 subjects. In NAFLD patients without microalbuminuria, the distribution was as follows: steatosis alone in 4 subjects, borderline NASH in 20 subjects, and definite NASH in 49 subjects ($P = .88$).

The presence of microalbuminuria in NAFLD patients was not associated with the degree of hepatic steatosis and necroinflammation. However, mean fibrosis scores were significantly higher in patients with microalbuminuria than in those without (1.27 ± 0.26 vs 0. 80 ± 0.11, $P < .05$). The same association was confirmed when microalbuminuria was treated as a continuous variable ($r = 0.256$, $P < .05$). Analysis of covariance, with corrections for age, sex, and HOMA-IR, showed significant differences in fibrosis scores according to the presence or absence of microalbuminuria ($F = 2.229$, $P < .05$), confirming the independence of this histologic difference from potential confounders, including insulin resistance.

Table 1
Characteristics of NAFLD patients with and without microalbuminuria

Factor	Microalbuminuria	No microalbuminuria	*P*
Patient number	14	73	–
Sex (male/female)	8/6	40/33	.87
Age (y)	45.7 ± 9.9	47.2 ± 8.5	.54
BMI (kg/m^2)	29.7 ± 2.1	31.4 ± 5.7	.27
Systolic blood pressure (mm Hg)	125.0 ± 12.4	126.6 ± 14.1	.36
Diastolic blood pressure (mm Hg)	74.3 ± 8.7	78.6 ± 10.7	.16
Triglycerides (mg/dL)	157 ± 77	162 ± 82	.81
HDL cholesterol (mg/dL)	44.6 ± 6.0	47.3 ± 10.9	.42
AST (IU/L)	48.3 ± 17.9	43.1 ± 17.5	.32
ALT (IU/L)	75.7 ± 24.2	69.9 ± 35.8	.56
Serum ferritin (ng/mL)	103 ± 78	114 ± 89	.37
HOMA-IR index	5.8 ± 3.7	3.9 ± 1.3	<.001
Simple steatosis/borderline NASH/definite NASH (no. of patients)	1/3/10	4/20/49	.88
Grading for steatosis	2.02 ± 0.26	1.91 ± 0.26	.44
Grading for necroinflammation	1.89 ± 0.83	1.78 ± 0.76	.32
Staging for fibrosis	1.27 ± 0.26	0.80 ± 0.11	<.001

4. Discussion

In the present study, there are 2 principal observations. First, patients with biopsy-proven NAFLD and microalbuminuria have higher insulin resistance as reflected by higher HOMA-IR values compared with NAFLD patients without microalbuminuria. Second, the presence of microalbuminuria independently predicts the severity of hepatic fibrosis among NAFLD patients even after adjustment for age, sex, BMI, AST, ALT, ferritin, hypertension, triglycerides, HDL cholesterol, and HOMA-IR. To the best of our knowledge, this is the first study investigating the clinical correlates of microalbuminuria in adult patients with biopsy-proven NAFLD.

In recent years, the relationship between kidney function and NAFLD has received increasing attention. Targher and colleagues [9] initially showed that the presence of NAFLD is associated with an increased prevalence of chronic kidney impairment in individuals with type 2 diabetes mellitus. Chang et al [13] have demonstrated that NAFLD predicts chronic kidney disease in nonhypertensive Korean men without diabetes. Hwang et al [8] have recently shown that NAFLD is associated with an increased frequency of microalbuminuria in persons with prediabetes and newly diagnosed diabetes, independent of baseline confounding variables.

Microalbuminuria reflects the increased passage of albumin through the glomerular filtration barrier [14]. Our findings that patients with NAFLD and microalbuminuria were more insulin resistant than those with a normal urinary albumin excretion are not surprising. Different mechanisms may link insulin resistance to abnormal albuminuria [15]. Insulin resistance and hyperinsulinemia have been associated with endothelial dysfunction, increased vascular permeability, and sustained glomerular hyperfiltration that can lead to increased albumin ultrafiltration and leakage into the urine. In addition, insulin resistance has been linked to mesangial hyperplasia, altered renal cellular metabolism, renal hypertrophy, and alterations in the renal matrix and inner medulla, effects that may directly contribute to progressive kidney damage [15]. Another possible factor linking NAFLD, microalbuminuria, and insulin resistance is the renin-angiotensin system [16]. This system may be involved in the development of insulin resistance and appears to promote hepatic fibrogenesis [17]. It is thus

feasible that activation of both systemic and local renin-angiotensin system in subjects with NAFLD patients can mediate the interdependency of insulin resistance and microalbuminuria in this patient group.

More interesting is the observation of an independent association between microalbuminuria and the extent of liver fibrosis among patients with NAFLD. Because a growing body of evidence supports the notion that NAFLD is a feature of the MetS [18], it is possible that the presence of microalbuminuria—a renal manifestation of the MetS—identifies a subgroup of NAFLD with a poorer metabolic profile at higher risk for liver fibrosis. Of note, however, this relation was independent from insulin resistance per se as assessed by the HOMA-IR. The causes of liver fibrosis and its progression in the MetS have not been determined. The mechanisms underlying the hepatic fibrogenic response are incompletely understood, although, as in other tissues, considerable experimental evidence supports a key role for transforming growth factor–β (TGF-β) [19]. Of interest, recent evidence has suggested that circulating levels of TGF-β are predictive of microalbuminuria and renal disease [20]. We thus speculate that levels of TGF-β may act as a molecular link between renal disease progression and the development of liver fibrosis in patients with NAFLD. Further pathophysiologic studies, however, are needed to confirm this possibility.

There are 2 main limitations that need to be mentioned in this study. First, our study was cross-sectional and therefore does not elucidate the causal relationships between microalbuminuria and the presence of liver fibrosis in patients with NAFLD. Second, the relatively small sample size limits the generalizability of our conclusions. Further studies are necessary to determine the potential utility of microalbuminuria in multiracial patients with NAFLD as well as in patients with persistently normal aminotransferases, and in following fibrosis progression.

In conclusion, our data suggest the presence of an independent relationship between the severity of insulin resistance and microalbuminuria in patients with NAFLD. In addition, microalbuminuria may identify NAFLD patients with a higher grade of fibrosis. Monitoring urinary albumin excretion during the natural course of NAFLD may potentially provide us with information on insulin resistance and possibly progression of hepatic fibrosis. Assessment of albuminuria is inexpensive and can represent a useful clinical approach by which to identify NAFLD subjects at higher metabolic risk.

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