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Gallstone Disease Does Not Predict Liver Histology in Nonalcoholic Fatty Liver Disease

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Background/Aims: We sought to examine whether the presence of gallstone disease (GD) in patients with biopsy-proven nonalcoholic fatty liver disease (NAFLD) is associated with liver fibrosis and histological nonalcoholic steatohepatitis (NASH) score. **Methods:** We included 441 Turkish patients with biopsy-proven NAFLD. GD was diagnosed in the presence of sonographic evidence of gallstones, echogenic material within the gallbladder with constant shadowing and little or no visualization of the gallbladder or absence of gallbladder at ultrasonography, coupled with a history of cholecystectomy. **Results:** Fifty-four patients (12.2%) had GD (GD+ subjects). Compared with the GD- subjects, GD+ patients were older, had a higher body mass index and were more likely to be female and have metabolic syndrome. However, GD+ patients did not have a higher risk of advanced fibrosis or definite NASH on histology. After adjustment for potential confounding variables, the prevalence of GD in NAFLD patients was not associated with significant fibrosis (≥2) (odds ratio [OR], 1.06; 95% confidence interval [CI], 0.53 to 2.21; p=0.68) or definite NASH (OR, 1.03; 95% CI, 0.495 to 2.12; p=0.84). **Conclusions:** The presence of GD is not independently associated with advanced fibrosis and definite NASH in adult Turkish patients with biopsy-proven NAFLD. **(Gut Liver 2014;8:313-317)**

Key Words: Gallstone disease; Non-alcoholic fatty liver disease; Nonalcoholic steatohepatitis; Fibrosis

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD)—defined as fat accumulation exceeding 5% to 10% by the weight of the liver in the absence of other causes of steatosis—is a common metabolic liver disorder which can be a precursor to cirrhosis and hepatocellular carcinoma.[1-3] NAFLD is currently considered as the hepatic manifestation of the metabolic syndrome (MS)[4,5]—although this association is not universal[6]—and insulin resistance represents its pathophysiological hallmark.[7,8] Similar to NAFLD, gallstone disease (GD) is highly prevalent in Western countries and has been pathogenetically linked with the MS.[9-11] Accordingly, the two conditions share a number of risk factors, including obesity, diabetes, dyslipidemia, and hyperinsulinemia.[12,13] Of note, the prevalence of nonalcoholic steatohepatitis (NASH) in GD has been reported to be as high as 18% in the morbid obese population.[14] In addition, insulin resistance occurs more commonly in concurrent NASH and GD.[14]

Recent years have witnessed an increased interest in the pathophysiological relationships between NAFLD and GD. Previous studies have shown that GD was a highly prevalent condition in NAFLD patients,[15-18] and that it may represent an independent risk factor for NAFLD.[19] In a large series of 524 Italian patients with biopsy-proven NAFLD, Fracanzani and colleagues demonstrated that the prevalence of GD progressively increased with advancing fibrosis and with the severity of necroinflammatory activity.[20] In an effort to replicate these findings in an independent and ethnically diverse study population, we sought to examine whether the presence of GD in patients with biopsy-proven NAFLD is associated with hepatic fibrosis and the histological NASH score.

MATERIALS AND METHODS

1. Study participants

The study population for this study was taken from a cohort

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of patients prospectively collected and followed up in four different Departments of Gastroenterology in Turkey. Therefore, this is a retrospective analysis of a group of patients with biopsy-proven NAFLD who were recruited prospectively. To be included in this study, patients with NAFLD should meet the following criteria:[21] 1) presence of steatosis affecting more than 5% of the total hepatocytes on hepatic histology; 2) history of alcohol consumption of less than 140 g/wk for males and less than 70 g/wk for females; and 3) absence of specific diseases that could lead to hepatic steatosis. Patients were excluded in presence of the following conditions: inflammatory diseases, anemia, hemochromatosis, Wilson disease, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, biliary obstruction, α-1 antitrypsin deficiency, ischemic cardiac or cerebrovascular disease, impaired renal function, or malignancies. Moreover, subjects using estrogens, amiodarone, steroids, tamoxifen, and lipid-lowering agents were not eligible for this cohort. All of the patients with a persistent (>6 months) elevation of transaminases and steatosis on ultrasound were considered as candidates for liver biopsy. Other potential candidates included 1) subjects with normal transaminases in presence of hepatomegaly and/or splenomegaly, and 2) subjects with normal transaminases but persistently increased γ-glutamyl transferase. After the exclusion of patients with possible bleeding disorders (based on personal and family history, complete blood count, prothrombin time, and activated partial thromboplastin time), liver biopsy was performed by an experienced gastroenterologist. After applying the inclusion and exclusion criteria, a total of 441 consecutive patients with biopsy-proven NAFLD (239 males and 202 females; mean age, 45.2±10.1 years) were available for this study. 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.

2. Clinical data collection

All subjects underwent a complete medical history and physical examination. Physical examination included measurements of height, weight, waist circumference, systolic blood pressure, and diastolic blood pressure. The body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. After 15 minutes of seated rest, blood pressure was measured twice from the right arm of the seated patient with an automated sphygmomanometer with 1 minute of rest between measurements. The average of the two measures was recorded. Subjects were instructed to fast overnight for 12 hours before venipuncture. Fasting laboratory assays included glucose, insulin, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides, alanine aminotransferase, aspartate aminotransferase, albumin, serum ferritin, and C-reactive protein. Diabetes mellitus was diagnosed according to American Diabetes Association criteria.[22] The MS was diagnosed using the Adult Treatment Panel III criteria.[23] Insulin resistance was defined according to the homeostatic metabolic assessment, using the following formula: insulin resistance=fasting plasma insulin (μU/mL)×fasting plasma glucose (mmol/L)/22.5.

3. Liver histology

Ultrasonography-guided liver biopsies were performed under conscious sedation using a 16-gauge Hepafix needle (Braun, Melsungen, Germany). Liver biopsies were processed routinely, and scored by a single pathologist in each participating center. To control for biopsy size, the length of the biopsy was measured with a hand ruler, and the number of portal areas on a cross-section was counted. The minimum number of portal areas in biopsy samples was 10. The severity of steatosis was graded 1 to 3 according to the percentage of cells with fatty droplets (1, 5% to 33%; 2, 33% to 66%; and 3, >66%). The stage of fibrosis was scored based on a 5-point scale, as follows: stage 0, absence of fibrosis; stage 1, perisinusoidal or portal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, septal or bridging fibrosis; and stage 4, cirrhosis. The diagnosis of NASH was based on the Brunt criteria, as modified by Kleiner *et al.*[24] The histological NASH score was defined as the unweighted sum of the scores for steatosis (0 to 3), lobular inflammation (0 to 3), and ballooning (0 to 2), thus ranging from 0 to 8. Cases with a NASH score of 0 to 2 were considered as simple steatosis, whereas those with scores of 3 or 4 were considered as borderline NASH. Cases with a score of 5 or higher were considered as definite NASH.

4. Definition of gallstone disease

According to previous methodology,[20] GD was diagnosed in the presence of sonographic evidence of gallstones, echogenic material within the gallbladder with constant shadowing and little or no visualization of the gallbladder, or absence of gallbladder at ultrasonography, coupled with a history of cholecystectomy.

5. Statistical analysis

Continuous data were tested for normality using the Shapiro-Wilk test and expressed as mean±SD (if the distribution was normal) or medians with interquartile ranges (in presence of skewed variables). Gaussian variables were analyzed with the Student t-test, whereas the Mann-Whitney U test was used for nonparametric measures. The Pearson chi-square test was used to test for differences in proportions. Two separate logistic regression analyses were constructed to assess the variables independently associated with 1) the presence of advanced fibrosis (≥2), and 2) the presence of definite NASH. All variables significant at univariate analyses were entered in the multivariable models. All calculations were performed using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL, USA). A value of

p<0.05 (two-sided) was considered statistically significant.

RESULTS

1. General characteristics of NAFLD patients with and without gallstone disease

Fifty-four patients of the 441 NAFLD patients (12.2%) had GD (GD+ subjects). Of them, 41 (77.7%) had a history of cholecystectomy for symptomatic GD. Compared with GD-, those who had GD+ were older, had a higher BMI, and showed a higher prevalence of female subjects and the MS (Table 1).

2. Liver histology

The mean liver biopsy length in our cohort was 2.7 cm (range, 1.8 to 3.4 cm). The histological findings are depicted in Table 2. There were no differences in steatosis, lobular inflammation, ballooning, portal inflammation, fibrosis stage, and NASH score between GD+ and GD- subjects. Significant fibrosis (≥2) was observed in 104 patients (23.5% of total); of them, 15 were GD+ (27.7%) and 89 were GD- (23.0%, p=0.54). Definite NASH was diagnosed in 283 patients (64.2% of total), that is, in 35 cases (64.8%) with GD+ and in 248 patients (64.1%) with GD- (p=0.91). Therefore, GD+ could not discriminate between definite NASH (n=283) versus borderline NASH (n=110) or simple steatosis (n=48).

3. Multivariable analysis

After adjustment for age, sex, BMI, and the prevalence of the MS (i.e., the factors significantly associated with GD in univari-

Table 2. Liver Histology in Patients with Biopsy-Proven Nonalcoholic Fatty Liver Disease with and without Gallstone Disease

Histology	GD+ (n=54)	GD- (n=387)	p-value
Steatosis	2 (1-3)	2 (1-2)	0.11
Lobular inflammation	2 (1-2)	2 (1-2)	0.67
Ballooning	2 (1-2)	1 (1-2)	0.94
Portal inflammation	0 (0-1)	0 (0-1)	0.75
Fibrosis stage	1 (0-2)	1 (0-1)	0.22
NASH score	5 (4-6)	5 (4-6)	0.37

Data are presented as median (interquartile range).
GD, gallstone disease; NASH, nonalcoholic steatohepatitis.

Table 1. General Characteristics of Patients with Biopsy-Proven Nonalcoholic Fatty Liver Disease with and without Gallstone Disease

Characteristic	GD+ (n=54)	GD- (n=387)	p-value
Sex, male/female	15/39	224/163	<0.001
Age, yr	48±8	44±10	0.008
Waist circumference, cm	102±9	103±9	0.61
Body mass index, kg/m^2	33.4±10.7	30.8±4.8	0.003
Glucose, mg/dL	116±40	107±33	0.08
Diabetes mellitus, yes/no	19/35	104/283	0.27
Smoking, pack/yr	1 (0-15)	1 (0-14)	0.43
HOMA-IR	4.2 (3.0-17.6)	4.0 (2.4-8.3)	0.34
Systolic blood pressure, mm Hg	129±18	126±16	0.17
Diastolic blood pressure, mm Hg	80±8	80±10	0.84
Metabolic syndrome, yes/no	41/13	229/158	0.03
AST, U/L	42 (30-64)	44 (34-59)	0.74
ALT, U/L	69 (43-98)	73 (52-102)	0.56
Total cholesterol, mg/dL	215±39	212±45	0.61
HDL-C, mg/dL	45±11	44±10	0.33
LDL-C, mg/dL	136±35	134±38	0.75
Triglycerides, mg/dL	159 (116-229)	160 (119-233)	0.92
Albumin, g/dL	4.6±0.4	4.7±0.4	0.31
Serum ferritin, mg/dL	101 (48-136)	96 (47-168)	0.55
Hemoglobin, g/dL	14.1±1.3	14.4±1.6	0.15
Glycated hemoglobin, %	6.0 (5.5-6.9)	5.9 (5.4-6.7)	0.33
C-reactive protein, mg/dL	0.56 (0.10-1.68)	0.59 (0.13-2.43)	0.63

Data are presented as mean±SD or median (interquartile range).
GD, gallstone disease; HOMA-IR, homeostasis model of insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

ate analysis), logistic regression analysis showed that the presence of GD in NAFLD patients was not associated neither with significant fibrosis (≥2) (odds ratio [OR], 1.06; 95% confidence interval [CI], 0.53 to 2.21; p=0.68) nor with the presence of definite NASH (OR, 1.03; 95% CI, 0.495 to 2.12; p=0.84). When the calculations were repeated using the presence of a history of cholecystectomy (symptomatic GD) as a potential predictor, we similarly found no association with liver histology (data not shown).

DISCUSSION

GD is the most common disorder of the gastrointestinal tract and it is strongly associated with metabolic risk factors.[9,10] Because hepatic steatosis is currently considered as the hepatic manifestation of the MS,[7,8] interest is mounting on the potential relationships between NAFLD and GD.[12-20] To our knowledge, this is one of the largest report to date focusing on the association between biopsy-proven NAFLD and GD. The results of this multicenter cross-sectional study conducted in Turkey indicated that patients with histology-proven NAFLD and GD were older, had a higher BMI, and showed a higher prevalence of female subjects and the MS compared with those without GD. However, we did not find any significant association of GD with neither liver fibrosis nor definite NASH both at univariate and multivariable analysis.

Our results differ significantly from those recently obtained by Fracanzani *et al.*,[20] who reported a highly significant independent association between the presence of GD and a higher risk of NASH and significant fibrosis in a large series of 524 Italian patients with NAFLD. However, in keeping with the findings by Fracanzani *et al.*,[20] we were able to confirm that GD is associated with female sex, obesity, and the MS. The discrepancy between our results and those of Fracanzani and colleagues[20] concerning the association between GD and liver histology in NAFLD cannot be easily explained. One possible explanation might be related to patient selection. First, the overall prevalence of GD was significantly higher in the paper by Fracanzani and coworkers[20] (20.0% vs 12.2% in our study). The prevalence of GD has been shown to vary in the general population from approximately 10% to 15% in the United States[25] to 9.5 to 18.9 in Italy.[26] Therefore, ethnic factors may potentially explain the differences between the studies. In addition, our patients are significantly younger and have a higher BMI compared with those of Fracanzani *et al.*[20] Based on their findings, Fracanzani and colleagues[20] suggested the opportunity of a routine liver biopsy at the time of cholecystectomy to establish an early diagnosis of NAFLD. Although NAFLD and GD certainly share several risk factors and frequently coexist,[18-20] our results indicate that the presence of GD does not invariably reflect a higher risk of definite NASH or advanced fibrosis. Therefore, further studies are needed to investigate the potential cost-effectiveness of routine liver biopsies to screen for advanced liver disease in the presence of sonographic evidence of gallstones or echogenic material within the gallbladder. It is also noteworthy that a significant genetic component contributes to the pathogenesis of both NAFLD[27,28] and GD.[9] In this regard, genetic polymorphisms of the human patatin-like phospholipase domain containing 3 gene have been strongly associated with the severity of NAFLD,[29,30] but no relation with the risk of GD was detected.[31] These results suggest that the genetic determinants of severe NAFLD and GD are likely different.

Several caveats of this study merit comment. First, our sample included subjects of Turkish nationality, so that results cannot be extrapolated to populations with different ethnic background. Second, the sample size was similar to that of the study by Francanzani and colleagues.[20] Given the discrepant findings, further studies in larger cohorts are needed to draw definite conclusions. Third, the histological evaluation was performed in each participating center by different pathologists. However, the intraobserver variation in grading and staging of the biopsy for each pathologist was low and this issue was likely to be noninfluential. Finally, because of the retrospective nature of our study, we were unable to differentiate between pigmented stones and cholesterol stones based only on ultrasonography. However, our patients did not have any risk factors for pigmented gallstones.

In summary, the results of our study confirm and expand previous findings showing that GD is associated with age, female sex, obesity, and the MS, but not with an increased risk of NASH and significant fibrosis in patients with NAFLD. Further studies involving a greater number of patients are warranted to investigate the potential clinical usefulness of liver biopsy to screen for advanced liver disease in the presence of sonographic evidence of gallstones or echogenic material within the gallbladder.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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