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Original Article

Impaired Gallbladder Motility and Increased Gallbladder Wall Thickness in Patients with Nonalcoholic Fatty Liver Disease

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Background/Aims
Nonalcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease worldwide. Along with the increase in the incidence of NAFLD and associated obesity, an increase in gallbladder disease (GD) has been noted. This has led to the identification of a new disease entity called fatty GD. There is a gap in the literature on the dynamics of gallbladder function in patients with NAFLD.

Methods
An observational case-control study, a total of 50 patients with biopsy proven NAFLD without gallbladder stone/sludge and 38 healthy comparison subjects were enrolled. Fasting, postprandial gallbladder volumes (PGV), gallbladder ejection fraction (GEF), and fasting gallbladder wall thickness (FGWT) were measured by real-time 2-dimensional ultrasonography.

Results
Fasting gallbladder wall thickness, fasting gallbladder volumes and PGV were significantly higher in patients with NAFLD than control subjects ($P < 0.001$, $P = 0.006$, and $P < 0.001$, respectively). Gallbladder ejection fraction was significantly lower in the NAFLD group than the controls ($P = 0.008$). The presence of NAFLD was an independent predictor for GEF, PGV, and FGWT. Also, steatosis grade was an independent predictor for GEF, and GEF was significantly lower in the nonalcoholic steatohepatitis (NASH) subgroup than the controls.

Conclusions
Gallbladder dysfunction and increase in gallbladder wall thickness exists in asymptomatic (without stone/sludge and related symptoms) patients with NAFLD and are useful in identifying fatty GD. Measurement of these variables in NAFLD patients may be useful in identifying those at higher risk for GD.
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Key Words
Gallbladder; Non-alcoholic fatty liver disease; Physiopathology

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease worldwide.[1,2] The prevalence of NAFLD is estimated to be between 20% and 30% in Western adults.[3,4] Abdominal obesity is an important etiological factor in the pathogenesis of NAFLD and recent reports have shown an association between increased gallbladder disease (GD) and cholecystectomy incidence and abdominal obesity.[5-8] This increase in GD may be linked to increasing frequency of obesity and metabolic syndrome (MetS). Additionally there have been various publications investigating an association between NAFLD and GD showing increased GD prevalence in NAFLD patients.[9-14] However there is no data available showing a causal relationship on this issue regarding gallbladder motility in patients with NAFLD.

The present study was designed to investigate the relationship between fasting gallbladder volumes, ejection fraction, residual volume, gallbladder thickness, biochemical values, histopathological parameters, and anthropometric measurements in patients with biopsy-proven NAFLD without GD and healthy controls.

Materials and Methods

Study Subjects

In this observational case-control study, a total of 50 patients with NAFLD (30 men and 20 women; mean age, 42.4 ± 9.7 years) without gallbladder stone/sludge and 38 healthy comparison subjects (22 men and 16 women; mean age, 40.1 ± 10.7 years) were enrolled. Patients evaluated in the gastroenterology clinic within the last 1 year with high transaminase levels, and with diagnosis of NAFLD in histopathological evaluation were included into the study. All patients had alanine aminotransferase elevations for at least 6 months; they had no history of hepatotoxic drug use, hormone replacement therapy or herbal products, and no alcohol use of more than 20 g/day. Viral serology, autoimmune markers, iron status, ceruloplasmin, serum and 24-hours urinary copper, alpha-1 antitrypsin levels, thyroid functions, and Kayser-Fleischer rings (with ophthalmological examination) were assessed. In order to exclude malignancy and/or cholestatic diseases, ultrasonographic examinations were performed. The patients having hepatosteatosis on ultrasonography (US) were followed for 6 months. An US-guided liver biopsy was performed on patients with high transaminase levels persisting after 6 months and those found to have NAFLD on histopathologic examination were enrolled in the study. The healthy control group subjects had no illnesses, alcohol use, drug or herbal substance use, and no history of previous liver diseases. They had negative viral hepatitis serology tests and normal liver US. This study was approved by the Istanbul Medeniyet University ethics committee (Approval number: 2015/0067, date: 27.05.2015.)

Clinical and Laboratory Evaluations

A complete physical examination was performed on all subjects. Anthropometric assessment of height and weight were recorded, body mass index (BMI, kg/m^2) was calculated, and waist circumference (cm) was measured. Blood pressures were obtained after ten minutes of rest in a quiet room. Venous blood samples were taken in the morning after a 12-hour fast. Complete blood counts and biochemical parameters were assessed using standard methods. The Adult Treatment Panel III[15] for MetS and American Diabetes Association[16] criteria was used for diabetes mellitus diagnosis. Homeostatic model assessment-insulin resistance index [fasting plasma insulin (mU/mL) × fasting plasma glucose (mg/dL)/405.23] were used for determining insulin resistance.

Ultrasonographic Evaluation

All ultrasonographic examinations and US guided percutaneous liver biopsies were performed by the same radiologist. After a 12 hour overnight fast, fasting and postprandial gallbladder volumes were measured sonographically before and 45 minutes after ingestion of a standard liquid test meal (33 g fat, 5 g protein, 59 g carbohydrate, 554 kcal per 100 mL). The thickness of the gallbladder wall, residual volume, and postprandial ejection fractions were calculated in all patients. Fasting and postprandial gallbladder volumes (FGV and PGV) and fasting and postprandial gallbladder wall thicknesses (FGWT and PGWT) were measured by real-time 2-dimensional ultrasonography (SSA-270A; Toshiba, Tokyo, Japan; with a 3.75 mHz curved transducer). Gallbladder dimensions in the longitudinal, transverse and sagittal planes were obtained. The smallest volume obtained after feeding at the 45th minute was determined as the postprandial (or residual) gallbladder volume. Gallbladder volumes (GV) and ejection fractions were determined using the following formulas; GV = π/6x (L x W x H) [V = gallbladder volume, L = sagittal length, W = width, H = axial height].[17] Gallbladder ejection fraction (GEF) = $(V_0–V_t)/V_0$ X100 [V_0 = FGV, V_t = PGV at 45th minute].

Histological Analysis

All of the biopsies were performed under US guidance. The

liver specimens were deemed sufficient if the length of the tissue was greater than 2 cm and/or showing more than 6 portal areas in histological examination. The liver specimens were stained with hematoxylin-eosin, Masson's trichrome, and reticulin silver stains. They were scored and evaluated by an experienced hepatopathologist blinded to the clinical status of the patients. Histological evaluation was done according to the NAFLD scoring system recommended by National Institute of Diabetes and Digestive and Kidney Diseases Nonalcoholic Steatohepatitis (NASH) Clinical Research Network.[18] Hepatic steatosis was graded from 1 to 3 according to the steatosis ratio as 5-33%, 33-66%, and > 66% representing score 1, 2, and 3, respectively. Lobular inflammation was defined as an overall assessment of all inflammation; no foci as score 0, < 2 foci per ×200 field as score 1, 2-4 foci per ×200 field as score 2, and > 4 foci per ×200 field as score 3. Ballooning scoring was defined as score 0 if there was no ballooning of hepatocytes, score 1 if there were few, and score 2 if there were numerous ballooning hepatocytes. Fibrosis was staged as follows: stage 0, no liver fibrosis; stage 1, perisinusoidal or periportal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, bridging fibrosis; and stage 4, cirrhosis. Histologically, the total NASH score was calculated as a sum of steatosis (1-3), lobular inflammation (0-3), and ballooning (0-2). It was graded according to the total NASH score 0-2 as simple steatosis, 3-4 as borderline NASH, 5 or greater were diagnosed as definitive NASH.[18]

Statistical Methods

Data was processed on a personal computer and analyzed using SPSS 16.0 (SSPS Inc, Chicago, IL, USA). Normally distributed continuous variables were presented as mean ± standard deviation; skewed continuous variables were characterized by the medians and interquartile ranges. The student *t* test was used in the evaluation of the difference between the 2 averages of the independent groups. Differences in the values of fasting gallbladder volumes, ejection fraction, residual volume and gallbladder thickness among the 4 groups were determined by one-way analysis of variance followed by the Bonferroni multiple-comparison post-hoc test. Categorical data were analyzed by using the χ^2 test. Spearman rank correlation was used to examine the relationship between variables. Multiple linear regression analysis was performed to evaluate the independence of the association between measurements related to gallbladder, clinical, biochemical, and histological parameters of liver injury in NAFLD patients. The covariates for these analyses were BMI, waist circumference, low density lipoprotein cholesterol, triglycerides, alanine aminotransferase, and histological steatosis scores. *P*-values < 0.05 were considered statistically significant.

Table 1. Clinical, Biochemical, and Radiological Characteristics of the Nonalcoholic Fatty Liver Disease Patients and Healthy Controls

	Healthy controls (n = 38)	NAFLD group (n = 50)	*P*-value
Gender (males/females)	22/16	30/20	> 0.05
Age (yr)	40.10 ± 10.70	42.40 ± 9.70	> 0.05
BMI (kg/m^2)	23.90 ± 5.10	31.10 ± 5.60	< 0.001
Waist circumference (cm)	78.70 ± 13.80	101.80 ± 9.50	< 0.001
Sedimentation (mm/hr)	18.20 ± 10.90	13.80 ± 9.60	> 0.05
C-reactive protein (mg/L)	3.80 ± 2.80	5.60 ± 4.40	0.042
White blood cells (×10^9/L)	6.11 ± 1.70	7.37 ± 2.50	0.009
HOMA-IR	0.94 ± 0.50	2.70 ± 1.80	< 0.001
Total cholesterol (mmol/L)	4.67 ± 0.90	5.26 ± 1.50	0.043
Triglycerides (mmol/L)	1.06 ± 0.60	2.31 ± 2.00	< 0.001
LDL cholesterol (mmol/L)	2.89 ± 0.80	3.57 ± 1.00	0.002
HDL cholesterol (mmol/L)	1.30 ± 0.30	1.18 ± 0.20	> 0.05
AST (U/L)	20.20 ± 6.10	39.70 ± 21.10	< 0.001
ALT (U/L)	17.30 ± 10.60	58.00 ± 37.00	< 0.001
MetS (%)	0	58	
Fasting gallbladder wall thickness (mm)	1.12 ± 0.38	1.48 ± 0.46	< 0.001
Fasting gallbladder volume (mL)	21.73 ± 11.10	27.86 ± 8.40	0.006
Postprandial gallbladder volume (mL)	10.73 ± 5.70	17.84 ± 8.18	< 0.001
Gallbladder ejection fraction (%)	48.00 ± 19.15	36.80 ± 19.30	0.008

NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; HOMA-IR, homeostatic model assessment-insulin resistance; LDL, low density lipoprotein; HDL, high density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MetS, metabolic syndrome.
Values are expressed as mean ± SE.

Results

The main clinical and laboratory characteristics and gallbladder kinetics of the patients and controls are described in Table 1. The age and gender distribution rates were similar between patients with NAFLD and controls. Ten (20%) patients had simple steatosis, 22 (44%) patients had borderline NASH, and 18 (36%) patients had definitive NASH in the NAFLD group. FGWT, FGV, and PGV were significantly higher in patients with NAFLD than control subjects ($P < 0.001$, $P = 0.006$, and $P < 0.001$, respectively). GEF was significantly lower in the NAFLD group than the controls ($P = 0.008$). There was no significant difference between patients with or without MetS among patients with NAFLD with respect to GEF (36.20 ± 20.30% and 37.00 ± 18.70%, respectively) and FGWT (1.53 ± 0.44 mm and 1.40 ± 0.51 mm, respectively).

Gallbladder kinetics of controls, patients with simple steatosis, and patients with (borderline and definitive) NASH (histological subgroups of NAFLD) are described in Table 2. Post-hoc analyses showed that FGWT (Fig. 1), FGV, and PGV were significantly higher in NASH subgroups of NAFLD than control subjects ($P < 0.001$, $P = 0.017$, and $P < 0.001$, respectively). But there was no significant difference between other subgroups for FGWT. For these subgroups the GEF measured 48.00 ± 19.00%, 38.40 ± 16.60%, and 36.10 ± 20.20%, respectively ($P = 0.023$). GEF was significantly lower in the NASH subgroup than the controls ($P = 0.023$), but was not significantly different between other subgroups (Fig. 2).

Linear stepwise regression analyzes showed that presence of NAFLD was an independent predictor of GEF, PGV, and FGWT in a multivariate model (Table 3). Also, steatosis grade was an independent predictor for GEF ($P = 0.001$) (Table 3). Also, there were significant correlation between FGV and presence of NAFLD, and BMI. However, only BMI was an independent predictor of FGV (Table 3).

Discussion

This is the first study to assess gallbladder motility of biopsy proven NAFLD patients. Results of our study show significant

Table 2. Gallbladder Kinetics of Controls, Patients with Simple Steatosis, and Patients with (Borderline and Definitive) Nonalcoholic Steatohepatitis

	Healthy controls	Simple steatosis	Nonalcoholic steatohepatitis	*P*-value
Fasting gallbladder wall thickness (mm)	1.12 ± 0.38	1.31 ± 0.37	1.52 ± 0.48	0.001
Fasting gallbladder volume (mL)	21.73 ± 11.10	27.36 ± 11.10	27.94 ± 8.55	0.019
Postprandial gallbladder volume (mL)	10.73 ± 5.70	16.80 ± 7.60	18.10 ± 8.50	< 0.001
Gallbladder ejection fraction (%)	48.00 ± 19.15	38.40 ± 16.60	36.10 ± 20.20	0.023

Values are expressed as mean ± SE.

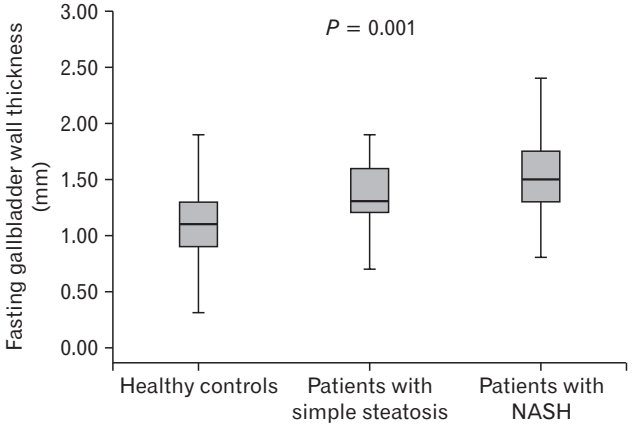

Figure 1. Fasting gallbladder wall thickness in healthy controls, patients with simple steatosis, and patients with nonalcoholic steatohepatitis (NASH).

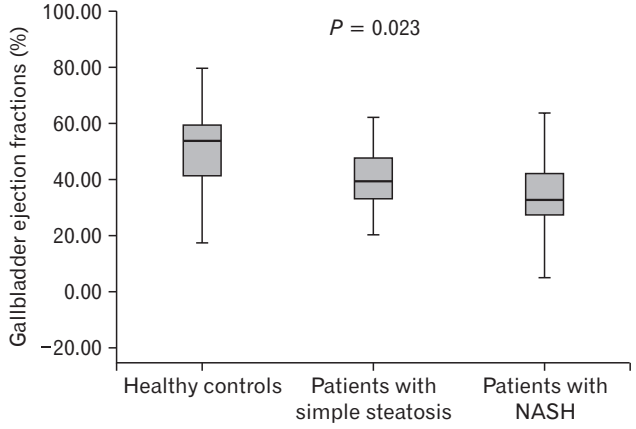

Figure 2. Gallbladder ejection fractions in healthy controls, patients with simple steatosis, and patients with nonalcoholic steatohepatitis (NASH).

Table 3. Results of the Correlations and Multiple Regression Analyze Between Gallbladder Dynamics and Relevant Parameters

	Correlations and correlation coefficients (*r*)	Independent predictors
Fasting gallbladder volume (mL)	The presence of NAFLD (*r*: 0.30) BMI (*r*: 0.36)	BMI (beta: 0.29, *t*: 2,33, *P*: 0.022)
Postprandial gallbladder volume (mL)	The presence of NAFLD (*r*: 0.45) BMI (*r*: 0.49) Age (*r*: 0.26) Steatosis grade (*r*: 0.34)	The presence of NAFLD (beta: 0.35, *t*: 2.4, *P*: 0.01) BMI (beta: 0.35, *t*: 2.66, *P*: 0.01) Steatosis grade (beta: 0.34, *t*: 2.61, *P*: 0.01)
Fasting gallbladder wall thickness (mm)	The presence of NAFLD (*r*: 0.39) BMI (*r*: 0.35) Steatosis grade (*r*: 0.3) Presence of DM-2 (*r*: 0.36)	The presence of NAFLD (beta: 0.33, *t*: 2.76, *P*: 0.03)
Gallbladder ejection fraction (%)	The presence of NAFLD (*r*: −0.28) BMI (−0.28) Age (*r*: −0.23) Steatosis grade (−0.41)	The presence of NAFLD (beta = −0.28, *t* = −2.26, *P*: 0.04) Steatosis grade (beta: −0.45, *t*: −3.4, *P*: 0.01)

NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; DM-2, diabetes mellitus type 2.

increase in FGWT, FGV, and PGV, and also reduced GEF in patients with NAFLD compared to control patients. Gallbladder motility is shown to decrease progressively in healthy controls, patients with simple steatosis, and patients with NASH: just as FGWT increases progressively in these same groups respectively. The results support an association between impaired gallbladder motility in patients with NAFLD and GWT.

Increased lipid presence in the gallbladder wall has been shown in patients with acalculous and calculous cholecystitis, and in recent years a link between increased acalculous cholecystitis and fatty GD has been suggested.[19] In patients with cholecystitis, ultrastructural lipid accumulation has been shown not only in macrophages, but also in the gallbladder wall (fibrocytes, muscle cells, and capillary endothelial cells).[20]

A possible mechanism explaining gallbladder dysfunction in patients with NAFLD is a theory called fatty GD that is described as increased fat deposition in the gallbladder wall. Obesity related fatty infiltration of the gallbladder leading to both structural and functional damage has been shown in animal studies.[21,22] One of the reasons of a possible relation between fatty GD and GD is the lipotoxicity caused by ectopic lipid accumulation in non-adipose tissues. The increase in cholesterol/phospholipid ratio in the muscle cells of the gallbladder due to this lipotoxicity decreases muscle contractility directly, and also decreases the humoral response to neurotransmitters like acetylcholine, cholecystokinin, and neuropeptide Y.[22-26] An important animal study on this subject has shown that ezetimibe, a cholesterol lowering agent limiting intestinal absorption of dietary and gallbladder originated cholesterol, both decreases lipid quantity of gallbladder wall and ameliorates gallbladder functions.[27] It also showed a positive effect on fatty GD by decreasing crystallization.

Another possible mechanism of reduced gallbladder motility in patients with NAFLD may be inflammatory damage caused by visceral adipose tissue related cytokines. It has been shown in numerous studies that adipose tissue releases various inflammatory molecules including TNF-alpha, IL-6, and cause end organ damage.[28-31] These inflammatory changes predispose to GD by impairing the muscle cell function of the gallbladder wall resulting from increased insulin resistance, and by impairing absorptive and secretive functions of the gallbladder.[32-34] Our results support this theory as FGV was found to be increased in patients with NAFLD, possibly due to the decreased absorptive function of gallbladder. A recent study by Pellegrinelli et al[33] has indeed shown that inflammation caused by human adipocytes causes decreased expression of contractile proteins in myotubes.[35]

Functional gallbladder disorder is defined as biliary pain resulting from poor gallbladder motility in the absence of microlithiasis, sludge, or gallstone disease. Otherwise, this disorder is considered a contributing risk factor for gallbladder sludge and stone. Patients with a GEF of less than 40 percent are considered to have poor gallbladder motility, and this result predicts which patients are likely to respond to cholecystectomy.[36] Additionally Sharma et al[37] showed that GEF is higher in microlithiasis patients than in gallstone patients, but lower than healthy volunteers. Our data showed that patients with NAFLD have reduced GEF (36.8%) and this result

may explain the increased GD in patients with NAFLD.

There are a few limitations that should be taken into account when interpreting the results, the low number of patients being the first. The study group consisted of only Turkish ethnicity, limiting generalization. Third, the exclusion of NAFLD in the control group was done by normal biochemical and ultrasonographic findings: no liver biopsies were performed on control subjects due to ethical concerns.

In conclusion, we have found increased impairment of gallbladder motility and increased FGWT in asymptomatic patients with biopsy proven NAFLD, compared to healthy controls. These data may explain the increased GD prevalence in NAFLD patients. Further clinical and translational research studies are needed to clarify these associations.

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Conflicts of interest: None.

Author contributions: Yasar Colak and Gulcin Bozbey performed the research; Yasar Colak and Ebubekir Senates designed the research study; Ebubekir Senates, Hasan Huseyin Mutlu, Banu Mesci, Mehmet Sait Doğan, Guralp Tasan, Feruze Yilmaz Enc, and Ilyas Tuncer contributed essential reagents or tools; Yasar Colak and Ebubekir Senates analyzed the data; and Yasar Colak, Tolga Erim, Ozge Telci Caklili, and Celal Ulasoglu wrote the paper.

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