Observational Study

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# The grade of nonalcoholic fatty liver disease is an independent risk factor for gallstone disease

## An observational Study

Young-Kyu Kim, MD, PhD[a,*], Oh-Sung Kwon, PhD[b], Kyu Hee Her, MD, PhD[a,*]

**Abstract**

There have been reports linking nonalcoholic fatty liver disease (NAFLD) with gallstone disease (GD) owing to shared risk factors. However, there are no reported associations between the different NAFLD grades and GD. This study aimed to determine whether NAFLD grade is an independent risk factor for GD in a Korean population.

This study enrolled 7886 participants who completed a questionnaire and underwent medical examination and ultrasound scanning at the Health Promotion Center of Jeju National University Hospital in Korea, from January 2009 to December 2017. Fatty liver grading and presence of gallstones were investigated using abdominal ultrasound. Body mass index and biochemical parameters were measured, and age, sex, and metabolic syndrome status were collected from medical records. Univariate and multivariate analyses were performed to identify risk factors for GD.

The estimated prevalences of NAFLD and GD were 40.6% and 4.5%, respectively. In the univariate analysis, factors associated with GD were age; NAFLD; presence of metabolic syndrome; and levels of fasting blood glucose, high-density lipoproteins, aspartate aminotransferase, and alanine aminotransferase. Multivariate logistic regression analysis revealed older age and higher NAFLD grade as independent risk factors for GD.

Older age and higher grade of NAFLD were independent risk factors for GD in our cohort. There was a strong correlation between grade of NAFLD on abdominal ultrasonography and GD.

**Abbreviations:** ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aminotransferase, BMI = body mass index, CAP = controlled attenuation parameter, GD = gallstone disease, GGT = gamma-glutamyltransferase, HDL = high-density lipoprotein, LDL = low-density lipoprotein, NAFLD = nonalcoholic fatty liver disease.

**Keywords:** Gallstones, Metabolic syndrome, Nonalcoholic fatty liver disease, Risk Factors

### 1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is defined clinically as hepatic steatosis, confirmed radiologically or pathologically, in the absence of excessive alcohol intake or other known chronic liver diseases. NAFLD has various histological features, from simple steatosis to nonalcoholic steatohepatitis or fibrosis, and can potentially progress to end-stage liver disease, cirrhosis, or liver cancer.[1] Furthermore, the consequences of NAFLD are not confined to liver-related morbidity and mortality, and the disease is closely associated with extrahepatic diseases, including cardiovascular diseases, chronic kidney disease, type 2 diabetes mellitus, and osteoporosis.[1–5] The biological mechanisms by which NAFLD leads to extrahepatic diseases have not fully established. However, cumulative evidence strongly indicates that peripheral resistance to insulin, dyslipidemia, and the activation of inflammatory pathways associated with NAFLD are relevant to the development of extrahepatic diseases.[6,7] Some studies have demonstrated that peripheral resistance to insulin and dyslipidemia are risk factors for gallstone disease (GD).[8,9] Thus, a relationship between GD and NAFLD is plausible because they share common risk factors. An estimated global prevalence of NAFLD is approximately 24%.[10] In Korea, the estimated prevalence of NAFLD is 25% to 30%, and this rate is steadily increasing because of Westernized dietary habits, excessive food intake, changes in lifestyle, an increase in the elderly population, and a general lack of exercise.[11]

GD is defined as the presence of stones in the gallbladder or common bile duct,[12] and it has become a more common diagnosis as ultrasonic examinations are more widely used alongside physical examination. Most patients with GD are asymptomatic, and only about 20% become symptomatic during 10 years of follow-up.[13] Nevertheless, some patients will eventually require treatment for symptomatic GD or acute cholecystitis. Previous studies have identified modifiable risk factors for GD, including NAFLD and metabolic syndrome.[7,14] Recently, an association between GD and NAFLD has been reported in a Chinese population[15]; however, an association

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between grade of NAFLD and GD could not be determined. This study aimed to determine whether NAFLD grade is an independent risk factor for GD in a Korean population.

## 2. Methods

### 2.1. Subjects

A total of 9207 people visited the Health Promotion Center of Jeju National University Hospital for medical checkups from January 2009 to December 2017. Among them, reasons for exclusion were previous cholecystectomy (n = 303) or hepatectomy (n = 4), refusal of consent or incomplete questionnaires (n = 692), and other hepatitis (n = 322). Finally, 7886 participants were included in the study. The study protocol was approved by the Institutional Review Board of Jeju National University Hospital. Informed consent was confirmed by the board.

### 2.2. Questionnaire

Each subject was asked to complete a questionnaire to collect demographic data and clinical indicators. The questionnaire was designed by the study investigators and included the following items and categories: telephone number, address, history of medical diseases (including specifically diabetes mellitus, hypertension, hyperlipidemia, heart disease, stroke, and tuberculosis, and related medication history), familial causes of death, smoking history, alcohol consumption, physical activity, and other medications.

### 2.3. Diagnosis of GD and grade of NAFLD

Specialist radiologists performed abdominal ultrasound examinations for all subjects using IU22 (Koninklijke Philips Electronics N.V., Amsterdam, the Netherlands) high-resolution ultrasound equipment. The abdominal ultrasound scans were performed after subjects had fasted for at least 8 hours. GD was identified based on the presence of echogenic and acoustic shadows and echo movement within the gallbladder depending on position change.[16]

NAFLD was defined according to the revised definition provided by the Korean Association for the Study of the Liver in 2013.[17] NAFLD is characterized by fatty infiltration of the liver on radiological examination or biopsy, without significant alcohol intake (<210 g/week for males and <140 g/week for females), medication intake causing fatty liver, or other causes (eg, autoimmune hepatitis, or hepatitis B antigen or hepatitis C virus antibody positivity). Accordingly, NAFLD was diagnosed on the basis of the brightness of the liver and the presence of diffuse echogenicity in the liver parenchyma on abdominal ultrasonography.

The grade of fatty liver was recorded as none (0), mild (1), moderate (2), or severe (3) according to the findings of liver brightness, hepatorenal echo contrast, deep attenuation of the ultrasound signal, and the blurring of vessels (Fig. 1).[18]

### 2.4. Definition of metabolic syndrome

According to the revised National Cholesterol Education Program criteria,[19] subjects may be diagnosed as having metabolic syndrome if they fulfill ≥3 of the following criteria: waist circumference 90 cm in males and 80 cm in females using the International Obesity Task Force criteria for the Asian-Pacific population to determine waist circumference[20]; triglycerides ≥150 mg/dL or antidyslipidemic medication use; high-density lipoprotein (HDL) <40 mg/dL in males and <50 mg/dL in females or antidyslipidemic medication use; blood pressure ≥130/85 mmHg or antihypertensive medication use; and fasting glucose ≥100 mg/dL or medication use (insulin or oral hypoglycemic agents).

### 2.5. Physical examination

Height and weight were automatically measured (GL-150R, G-Tech International Co., Gyeong-gido, Korea). Participant age and sex were collected from the medical records. Venous blood samples were taken after 8 hours of fasting. Fasting blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total cholesterol, triglycerides, HDL, and low-density lipoprotein (LDL) levels were measured using venous blood samples.

The prevalence of GD was calculated according to sex, study year, and age. The subjects were divided into 4 groups according to age: the <50, 50 to 59, 60 to 69, and ≥70-year age groups. Body mass index (BMI) was calculated by dividing weight by the square of height and classified into 4 groups, according to the World Health Organization's BMI for Asian populations classification[21]: underweight, <18.5 kg/m$^2$; normal weight, 18.5 to 22.9 kg/m$^2$; overweight, 23.0 to 24.9 kg/m$^2$; and obese, ≥25.0 kg/m$^2$. Fasting blood glucose was classified into 3 groups based on the standard proposed by the American Diabetes Association in 2015[22]: normoglycemia, <100 mg/dL; impaired fasting glucose, 100 to 125 mg/dL; and diabetes, ≥126 mg/dL. Fasting was defined as no caloric intake for at least 8 hours. Total cholesterol was classified into 3 groups: <200 mg/dL, 200 to 239 mg/dL, and ≥240 mg/dL. Serum LDL levels were classified into 5 groups: <100 mg/dL, 100 to 129 mg/dL, 130 to 159 mg/dL, 160 to 189 mg/dL, and ≥190 mg/dL. Serum HDL levels were classified into 3 groups: <40 mg/dL, 40 to 60 mg/dL, and ≥60 mg/dL. Serum triglyceride levels were classified into 4 groups: <150 mg/dL, 150 to 199 mg/dL, 200 to 499 mg/dL, and ≥500 mg/dL. Each lipid was classified according to the 2015 Korean Guidelines for Management of Dyslipidemia.[23] AST levels were considered elevated if they were >32 IU/L for males and >26 IU/L for females. ALT levels were considered elevated they were over 34 IU/L for males and over 24 IU/L for females.[24] ALP and GGT levels were considered high if they were >130 IU/L and 71 IU/L, respectively.

### 2.6. Statistical analysis

The clinical variables were compared using $\chi^2$ tests for categorical variables and Student *t* tests for continuous variables according to the presence of GD. Binary logistic regression analysis was performed to assess risk factors for GD, including age, sex, grade of fatty liver disease, BMI, fasting blood glucose, total cholesterol, LDLs, HDLs, triglycerides, AST, ALT, GGT, and ALP. Stepwise logistic regression was applied for the development of a fitted model estimating the predictive probability of GD when the factors were <0.1 on the univariate analysis by binary logistic regression analysis. A *P* value of <.05 was considered statistically significant. All statistical analyses were performed using SPSS version 18 (SPSS Inc., Chicago, IL).

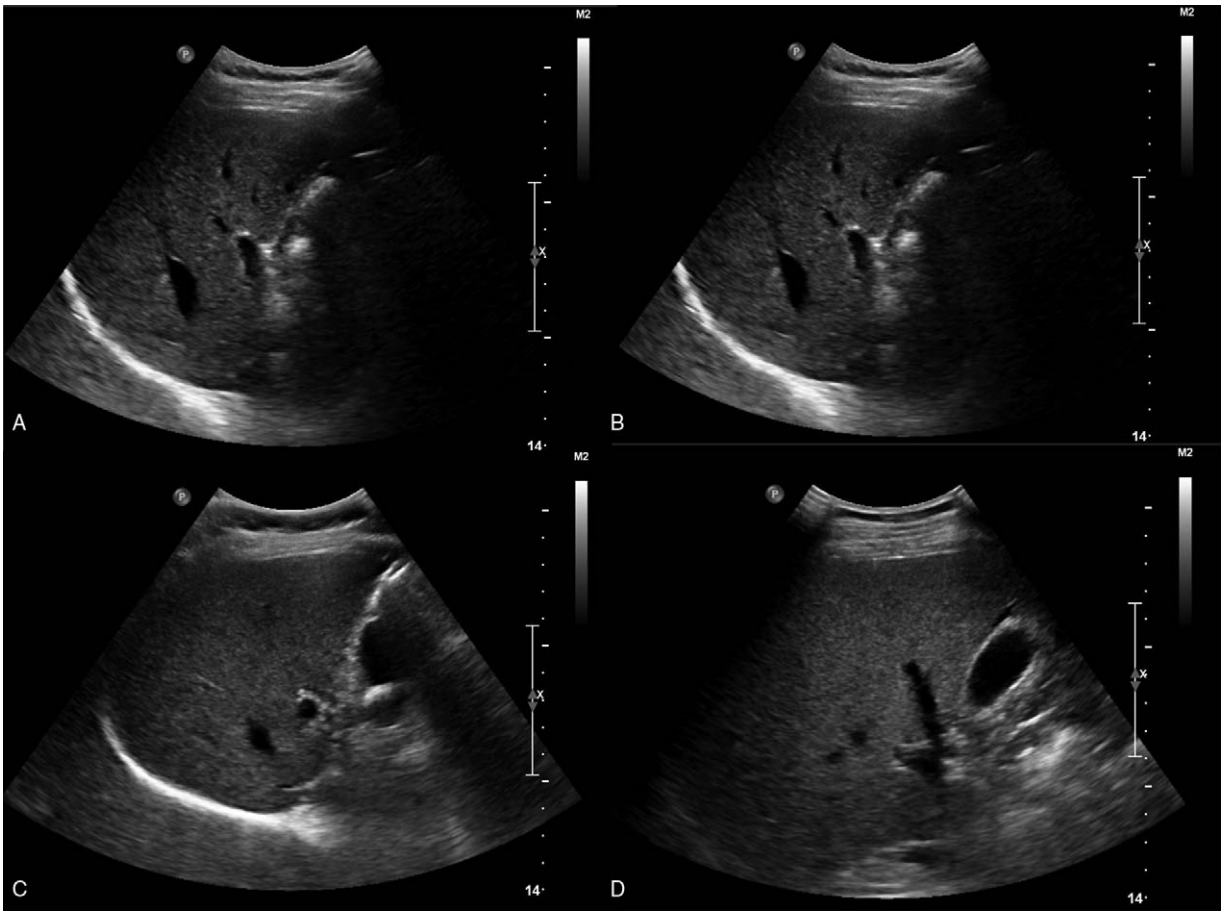


**Figure 1.** Fatty liver was graded according to sonographic findings. (A) Normal liver echogenicity; (B) mildly fatty liver with diffusedly increased liver echogenicity and appreciable periportal and diaphragmatic echogenicity; (C) moderately fatty liver with diffusely increased hepatic echogenicity obscuring periportal echogenicity, and diaphragmatic echogenicity is appreciable; (D) severely fatty liver in the diaphragmatic outline is obscure. Grade I: diffusely increased hepatic echogenicity with appreciable periportal and diaphragmatic echogenicity. Grade II: diffusely increased hepatic echogenicity obscuring periportal echogenicity, but diaphragmatic echogenicity is still appreciable. Grade III: diffusely increased hepatic echogenicity obscuring periportal as well as diaphragmatic echogenicity.

## 3. Result

### 3.1. Prevalence and correlation of GD and NAFLD

Of the 7886 participants, 4313 (54.7%) were males, 3573 (45.3%) were females. The overall prevalence of GD was 4.5% (n = 355). There was no correlation between the study year and the prevalence of GD ($r = -0.007$, $P = .516$). The overall prevalence of NAFLD was 40.6% (n = 3201), and the annual prevalence was lowest in 2009 (30.8%), compared with the highest in 2017 (53.9%) (Fig. 2). The annual percentage of the participants who were diagnosed with NAFLD was significantly correlated with the study period ($r = 0.040$, $P < .001$). Grade of NAFLD was positively correlated with the development of GD ($r = 0.550$, $P < .001$).

### 3.2. Comparison of clinical variables between participants with and without GD

The participants were divided into 2 groups according to the presence of GD or not. Mean age, BMI, fasting blood glucose, and ALP were significantly higher among participants with GD. Mean HDL level was significantly lower among participants with GD. Participants with GD had significantly higher rates of high-grade (grade 2–3) NAFLD, metabolic syndrome, and medication use for diabetes, hypertension, and dyslipidemia than those without GD (Table 1).

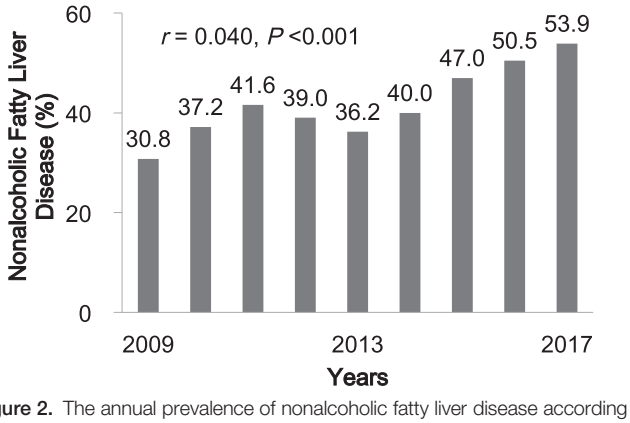


**Figure 2.** The annual prevalence of nonalcoholic fatty liver disease according to study years.

**Table 1**

**Comparisons of the variables according to the presence or absence of gallstones in participants who underwent medical checkups.**

| Variables | With GD (n = 355) | Without GD (n = 7531) | P |
|---|---|---|---|
| Sex | | | .254 |
| Male | 201 (56.6) | 4112 (54.6) | |
| Female | 154 (43.4) | 3419 (45.4) | |
| Grade of fatty liver disease | | | <.001 |
| 0 | 179 (50.4) | 4506 (59.8) | |
| 1 | 88 (24.8) | 1891 (25.1) | |
| 2 | 76 (21.4) | 1020 (13.5) | |
| 3 | 12 (3.4) | 114 (1.5) | |
| Metabolic syndrome | | | .001 |
| Yes | 112 (31.8) | 1822 (24.5) | |
| No | 240 (68.2) | 5625 (75.5) | |
| Age, y | 59.9 ± 11.6 | 55.8 ± 11.5 | <.001 |
| Body mass index, kg/m$^2$ | 25.6 ± 4.1 | 24.9 ± 3.7 | .002 |
| Fasting blood glucose, mg/dL | 102.1 ± 28.2 | 98.8 ± 29.6 | .041 |
| Total cholesterol, mg/dL | 198.6 ± 36.7 | 198.9 ± 37.2 | .896 |
| LDL-cholesterol, mg/dL | 122.3 ± 33.8 | 121.1 ± 34.3 | .531 |
| HDL-cholesterol, mg/dL | 52.0 ± 13.6 | 54.1 ± 13.7 | .006 |
| Triglycerides, mg/dL | 121.8 ± 76.0 | 118.8 ± 91.0 | .538 |
| AST, IU/L | 29.7 ± 40.5 | 27.7 ± 49.5 | .452 |
| ALT, IU/L | 32.1 ± 38.3 | 29.8 ± 70.3 | .552 |
| GGT, IU/L | 48.1 ± 64.4 | 45.2 ± 73.3 | .467 |
| ALP, IU/L | 220.2 ± 83.3 | 208.2 ± 86.9 | .016 |
| Medication for diabetes | | | .040 |
| Yes | 28 (7.9) | 397 (5.3) | |
| No | 327 (92.1) | 7134 (94.7) | |
| Medication for dyslipidemia | | | .571 |
| Yes | 11 (3.1) | 293 (3.9) | |
| No | 344 (96.9) | 7238 (96.1) | |
| Medication for hypertension | | | <.001 |
| Yes | 86 (24.2) | 1254 (16.7) | |
| No | 269 (75.8) | 6277 (83.3) | |

Values are expressed as n (%) or mean ± standard deviation. ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, GD = gallstone disease, GGT = gamma-glutamyltransferase, HDL = high-density lipoprotein, LDL = low-density lipoprotein.

### 3.3. Univariate analysis of risk factors for GD

The factors affecting GD are summarized in Table 2. The prevalence of GD was 3.1% in the 20- to 49-year age group, 3.7% in 50- to 59-year age group, 6.0% in the 60- to 69-year age group, and 6.9% in the ≥70-year age group. There was a significantly positive correlation between age and GD ($r = 0.074$, $P < .001$). Age; NAFLD grade; presence of metabolic syndrome; levels of fasting blood glucose, HDLs, AST, and ALT; medication use for diabetes and hypertension were significantly associated with GD.

### 3.4. Multivariate analysis of risk factors for GD

Binary logistic regression analysis was performed for clinical variables, including sex and medication intake for dyslipidemia (which were not significantly associated with GD in the univariate analysis), to adjust for risk factors affecting GD (Table 3). Age and grade of fatty liver disease were independent risk factors affecting GD. The prevalence of GD significantly increased with age (odds ratio [OR], 1.175 for the 50–59-year age group; OR, 2.000 for the 60–69-year age group; OR, 2.444 for the ≥70-year age group; $P = .002$) and NAFLD grade (OR, 1.480 for grade 1; OR, 1.860 for grade 2; OR, 3.105 for grade 3; $P < .001$).

### 3.5. Comparison of clinical variables between 2 groups according to grade of fatty liver disease

Participants were divided into 2 groups (grade 0–1 versus grade 2–3) according to grade of fatty liver on abdominal ultrasonography because those with grade 1 NAFLD tended to have similar clinical variables to one another (Table 4). The grade 2–3 group had significantly lower proportions of females and participants taking medication for diabetes, dyslipidemia, and hypertension; this group also had higher prevalences of metabolic syndrome and GD. There were significant differences in mean BMI, fasting glucose level, total cholesterol, LDL level, HDL level, triglycerides, AST, ALT, ALP, and γ-GTP between the 2 groups. Interestingly, mean age was not significantly different between the 2 groups.

## 4. Discussion

Among 7886 participants who underwent abdominal ultrasonography, 3201 (40.6%) scans revealed radiological findings of NAFLD, and 355 (4.5%) showed findings of gallstones. The current estimated prevalences of NAFLD in Western and Asian countries are 24% to 42% and 25% to 48%, respectively.[10,25,26] The estimated prevalence of NAFLD in this study is 40.6%, which is high compared to other studies conducted in Korea.[11,27] A reasonable explanation for this discrepancy is that the dietary and alcohol consumption habits of subjects in this study were a little bit different from other study populations. We previously reported that people from Jeju tend to consume more carbohydrates and alcohol compared with people living in mainland Korea.[9] Therefore, people from Jeju have higher mean fasting glucose levels, blood lipids, and BMI than people from the mainland. These observations might explain why this study population has a higher prevalence of NAFLD.

Female sex is classically a strong risk factor affecting GD, and most previous studies have reported a higher prevalence of GD among females than males.[12] However, in this study, females did not show a significantly higher prevalence of GD than males. Some authors have reported that female sex was not found to be a risk factor for GD in studies conducted on Korean populations.[9,28] One explanation given was that female sex was strongly affecting gallstone formation; the gap narrowed following menopause after which males started to catch up. Eventually, the overall prevalence of GD between males and females did not reach statistical significance. Kim et al[28] reported that the prevalence of GD was significantly higher among females younger than 40 years than among males. However, the GD prevalence among males increased as age increased past 50 years. Our unpublished data tended to coincide with this finding ($P < .067$ in participants' age <40 years and $P < .124$ in participants' age >50 years). The higher prevalence of GD among females younger than 40 years was likely to be related to the estrogen effect or pregnancy, whereas the higher prevalence of GD among males older than 50 years than among females older than 50 reflects the diminished effect of estrogen and pregnancy among females as well as lithogenic factors—such higher BMI, lower HDL-cholesterol and peripheral resistance to insulin—among males.[12,28] Therefore, although female sex strongly influenced the prevalence of GD, the overall difference in

**Table 2**

**Univariate analysis of risk factors for gallstone disease in participants who underwent medical checkups.**

| Factors | n | Number of gallstone disease, n (%) | Odds ratio (95% confidence interval) | *P |
|---|---|---|---|---|
| Age, y | | | | <.001 |
| 20–49 | 2380 | 75 (3.2) | 1.000 | |
| 50–59 | 2576 | 95 (3.7) | 1.177 (0.865–1.601) | .300 |
| 60–69 | 1919 | 115 (6.0) | 1.959 (1.455–2.638) | <.001 |
| ≥70 | 1011 | 70 (6.9) | 2.286 (1.636–3.194) | <.001 |
| Sex | | | | .455 |
| Male | 4313 | 201 (4.7) | 1.000 | |
| Female | 3573 | 154 (4.3) | 0.912 (0.743–1.142) | |
| Grade of fatty liver disease | | | | <.001 |
| 0 (None) | 4685 | 179 (3.8) | 1.000 | |
| 1 (Mild) | 1979 | 88 (4.4) | 1.171 (0.903–1.520) | .234 |
| 2 (Moderate) | 1096 | 76 (6.9) | 1.876 (1.422–2.474) | <.001 |
| 3 (Severe) | 126 | 12 (9.5) | 2.650 (1.435–4.893) | .002 |
| Metabolic syndrome | | | | .002 |
| Yes | 1934 | 112 (5.8) | 1.441 | |
| No | 5865 | | 1.000 | |
| BMI, kg/m$^2$ | | | | .237 |
| <18.5 | 149 | 4 (2.7) | 1.000 | |
| 18.5–22.9 | 2052 | 80 (3.9) | 1.471 (0.531–4.071) | .458 |
| 23–24.9 | 1850 | 78 (4.2) | 1.596 (0.576–4.420) | .369 |
| ≥25 | 3638 | 177 (4.9) | 1.854 (0.679–5.064) | .229 |
| Fasting blood glucose, mg/dL | | | | .010 |
| <100 | 5365 | 216 (4.0) | 1.000 | |
| 100–125 | 1818 | 97 (5.3) | 1.344 (1.051–1.718) | .018 |
| ≥126 | 703 | 42 (6.0) | 1.515 (1.078–2.129) | .017 |
| Total cholesterol, mg/dL | | | | .560 |
| <200 | 4172 | 191 (4.6) | 1.000 | |
| 200–239 | 2677 | 124 (4.6) | 1.012 (0.803–1.276) | .917 |
| ≥240 | 1037 | 40 (3.9) | 0.836 (0.591–1.184) | .314 |
| LDL-cholesterol, mg/dL | | | | .829 |
| <100 | 2047 | 91 (4.4) | 1.000 | |
| 100–129 | 2726 | 118 (4.3) | 0.973 (0.735–1.286) | .845 |
| 130–159 | 1980 | 93 (4.7) | 1.059 (0.788–1.424) | .702 |
| 160–89 | 726 | 30 (4.7) | 0.926 (0.608–1.412) | .723 |
| ≥190 | 224 | 13 (5.8) | 1.324 (0.728–2.409) | .357 |
| HDL-cholesterol, mg/dL | | | | .007 |
| <40 | 1032 | 65 (6.3) | 1.000 | |
| 40–60 | 4416 | 196 (4.4) | 0.691 (0.517–0.923) | .012 |
| ≥60 | 2438 | 94 (3.9) | 0.597 (0.431–0.826) | .002 |
| Triglyceride, mg/dL | | | | .657 |
| <150 | 5994 | 265 (4.4) | 1.000 | |
| 150–199 | 950 | 48 (5.1) | 1.150 (0.839–1.577) | .384 |
| 200–499 | 888 | 41 (4.6) | 1.046 (0.747–1.465) | .791 |
| ≥500 | 54 | 1 (1.9) | 0.480 (0.056–2.9761) | .375 |
| AST, IU/L | | | | .013 |
| ≤32 for males or ≤26 for females | 5425 | 223 (4.1) | 1.000 | |
| >32 for males or >26 for females | 2461 | 132 (5.4) | 1.322 (1.060–1.649) | |
| ALT, IU/L | | | | .011 |
| ≤34 for males or ≤24 for females | 4752 | 191 (4.0) | 1.000 | |
| >34 for males or >24 for females | 3134 | 164 (5.2) | 1.319 (1.065–1.633) | |
| GGT, IU/L | | | | .310 |
| ≤71 | 6765 | 298 (4.4) | 1.000 | |
| >71 | 1121 | 57 (5.1) | 1.163 (0.869–1.555) | |
| ALP, IU/L | | | | .382 |
| ≤130 | 497 | 19 (3.8) | 1.000 | |
| >130 | 6390 | 299 (4.7) | 1.235 (0.770–1.982) | |
| Medication for diabetes | | | | <.001 |
| Yes | 425 | 28 (6.6) | 1.000 | |
| No | 7461 | 327 (4.4) | 0.710 (0.436–0.969) | |
| Medication for dyslipidemia | | | | .449 |
| Yes | 304 | 11 (3.6) | 1.000 | |
| No | 7582 | 344 (4.5) | 1.038 (0.687–2.334) | |
| Medication for hypertension | | | | <.001 |
| Yes | 1340 | 86 (6.4) | 1.000 | |
| No | 6546 | 269 (4.1) | 0.690 (0.467–0.803) | |

Values are expressed as n (%) or mean ± standard deviation. ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, GD = gallstone disease, GGT = gamma-glutamyltransferase, HDL = high-density lipoprotein, LDL = low-density lipoprotein.

* This value was obtained using the binary regression test.

**Table 3**

**Multivariate analysis of risk factors for gallstone disease in participants who underwent medical checkups.**

| Factors | Odds ratio | 95% Confidence interval | *P* |
|---|---|---|---|
| Age, y | | | <.001 |
| 20–49 | 1.000 | | |
| 50–59 | 1.175 | 0.861–1.604 | .309 |
| 60–69 | 2.000 | 1.480–2.704 | <.001 |
| ≥70 | 2.444 | 1.741–3.431 | <.001 |
| Grade of fatty liver disease | | | <.001 |
| 0 | 1.000 | | |
| 1 | 1.480 | 0.875–1.485 | .331 |
| 2 | 1.860 | 1.406–2.460 | <.001 |
| 3 | 3.105 | 1.671–5.769 | <.001 |
| Medication for dyslipidemia | | | |
| Yes | 1.000 | | |
| No | 0.525 | 0.275–1.002 | .051 |

**Table 4**

**Comparisons of the variables according to grade of nonalcoholic fatty liver disease in participants who underwent medical checkups.**

| | Grade of fatty liver disease | | |
|---|---|---|---|
| | Grade 0–1 (n = 6664) | Grade 2–3 (n = 1222) | *P* |
| Sex | | | <.001 |
| Male | 3454 (51.8) | 859 (70.3) | |
| Female | 3210 (48.2) | 363 (29.7) | |
| Gallstone disease | | | <.001 |
| Yes | 267 (4.0) | 88 (7.2) | |
| No | 6397 (96.0) | 1134 (92.8) | |
| Metabolic syndrome | | | <.001 |
| Yes | 1238 (18.8) | 696 (57.6) | |
| No | 5352 (81.2) | 513 (42.4 | |
| Age, y | 56.0 ± 11.6 | 56.0 ± 11.1 | .965 |
| Body mass index, kg/m$^2$ | 24.6 ± 3.7 | 26.7 ± 3.7 | <.001 |
| Fasting blood glucose, mg/dL | 97.2 ± 28.3 | 108.8 ± 34.2 | <.001 |
| Total cholesterol, mg/dL | 197.0 ± 36.6 | 209.1 ± 39.0 | <.001 |
| LDL-cholesterol, mg/dL | 120.2 ± 33.3 | 126.3 ± 38.4 | <.001 |
| HDL-cholesterol, mg/dL | 55.2 ± 13.7 | 47.0 ± 10.9 | <.001 |
| Triglycerides, mg/dL | 107.9 ± 77.9 | 179.4 ± 123.7 | <.001 |
| AST, IU/L | 26.8 ± 51.5 | 33.3 ± 32.6 | <.001 |
| ALT, IU/L | 27.1 ± 68.1 | 45.3 ± 73.2 | <.001 |
| γ-GTP, IU/L | 41.4 ± 70.3 | 67.0 ± 82.8 | <.001 |
| ALP, IU/L | 207.2 ± 89.1 | 217 ± 72.4 | <.001 |
| Medication for diabetes | | | <.001 |
| Yes | 289 (4.3) | 136 (11.1) | |
| No | 6375 (95.7) | 1086 (88.9) | |
| Medication for hypertension | | | <.001 |
| Yes | 1026 (15.4) | 314 (25.7) | |
| No | 5638 (86.1) | 908 (74.3) | |
| Medication for dyslipidemia | | | <.001 |
| Yes | 232 (3.5) | 72 (5.9) | |
| No | 6432 (96.5) | 1150 (94.1) | |

Values are expressed as n (%) or mean ± standard deviation. ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = gamma-glutamyltransferase, HDL = high-density lipoprotein, LDL = low-density lipoprotein.

GD prevalence between males and females did not reach statistical significance.

This study demonstrated a positive correlation between the study time and the annual percentage of the participants who diagnosed with NADLF but not of GD. NAFLD could more easily be affected by a Westernized diet, excessive food intake, and changes in lifestyle (including a general lack of exercise) than GD. In other words, compared with NAFLD, there is a longer lag period between exposure to risk factors and the development of GD.

Age is a well-known risk factor for GD. In this study, older age was found to be an independent risk factor for GD. It has been reported that the cholesterol saturation of bile increases with age as a consequence of enhanced hepatic secretion of cholesterol and decreased bile acid synthesis. The progressive change in the ratio between bile acid synthesis and cholesterol saturation causes supersaturation of cholesterol.[29]

Previous reports have suggested a correlation between GD and NAFLD.[15] In this study, we showed a strong correlation between grade of NAFLD on abdominal ultrasonography and the presence of GD. Participants in the high-grade (grade 2–3) NAFLD group had worse blood lipid profiles, were more commonly obese, had a higher mean fasting blood glucose level, and a higher percentage of individuals taking medication for hypertension—all of which concur with other studies[15,30,31] and all of which are common risk factors for both NAFLD and GD.

All of the above-mentioned conditions—hypertriglyceridemia, obesity, peripheral resistance to insulin or diabetes, and hypertension—are closely related with metabolic syndrome,[7,14,31] and NAFLD is known to be significantly associated with metabolic syndrome. However, in this study, metabolic syndrome was not found to be an independent risk factor for GD in the final binary logistic regression model, even though it was strongly associated with the development of GD on univariate analysis. A possible explanation is that metabolic syndrome might be implicated only indirectly in the development of GD.

Many observational studies have demonstrated that NAFLD is associated with extrahepatic diseases, including cardiovascular disease, type 2 diabetes mellitus, chronic kidney disease, and osteoporosis.[1–6] However, the biological and genetic pathways associated with the influence of NAFLD on extrahepatic diseases remain unclear.[1,6,26] We do know, however, that peripheral resistance to insulin, pro-inflammatory mediators, oxidative stress, and lipotoxicity has complex associations with the development of extrahepatic diseases.[1,6,26] This study showed a significant relationship between the grade of NAFLD and GD. Participants with GD are more likely to have dyslipidemia, hyperglycemia, obesity, and metabolic syndrome, which have all been associated with NAFLD. Therefore, GD should be understood as a kind of extrahepatic disease in patients with NAFLD.

NAFLD prevalence has been continuously increasing in recent decades, and it has become a common disease. Therefore, physicians frequently encounter NAFLD patients. Additionally, NAFLD is clinically relevant to the development of extrahepatic diseases,[1,6] including GD.[3] Physicians should pay special attention to patients with NAFLD and provide them with information about extrahepatic diseases, including GD. Specific lifestyle modifications (ie, weight loss, smoking cessation, calorie-restricted diet, and increasing physical activity) should be emphasized for NAFLD patients, and physicians should consider prescribing aggressive pharmaceutical modifications in NAFLD

patients with metabolic syndrome or type 2 diabetes mellitus to decrease the morbidity, mortality, and medical expenses associated with extrahepatic diseases,[26] including GD.

Ultrasound shows a bright echo pattern in fatty liver and is widely used for screening for hepatic steatosis. However, some reports have shown that ultrasound cannot be used to precisely estimate the extent of steatosis. This limitation can be overcome using the controlled attenuation parameter (CAP) feature, which has been recently developed to quantify ultrasound attenuation during the measurement of liver stiffness vibration-controlled elastography.[32–34] CAP measurement is advantageous because it is an easy and fast examination providing a numerical value that correlates with the histological degree of steatosis. Regretfully, our institution was first equipped with CAP in March 2017, making it available for clinical use starting from July 2017. We could not have planned to use CAP on study participants because CAP was unavailable during the study period. Therefore, prospective studies about the influence of NAFLD grades (as measured by CAP) on GD prevalence will be required to support our findings.

This study had some limitations. First, it was carried out at a single institution. Furthermore, most of the subjects came from Jeju Island, which is located about 50 miles south of mainland Korea, and mainland Koreans were largely underrepresented in our study. Therefore, a multicenter study will be conducted on the mainland in the future. Second, NAFLD was only defined by ultrasonographic liver brightness and the presence of diffuse echogenicity in the hepatic parenchyma. It should be noted, however, that the diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver could be acceptable for meta-analyses.[35] Third, the participants could not be analyzed according to the type of gallstone (pigment or cholesterol stones), because this information was not included in the available medical records.

In conclusion, older age and higher grade of NAFLD were independent risk factors for GD in this cohort. There was a strong correlation between grade of NAFLD on abdominal ultrasonography and the presence of GD.

## Author contributions

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