

Nonalcoholic steatohepatitis in nonalcoholic fatty liver disease patients of Bangladesh

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Received: February 17, 2013 Revised: April 30, 2013

Accepted: May 8, 2013

Published online: May 27, 2013

Abstract

AIM: To explore the prevalence and risk factors for nonalcoholic steatohepatitis (NASH) in nonalcoholic fatty liver disease (NAFLD) patients.

METHODS: We have included 493 patients with sonographic evidence of a fatty change, and 177 of these individuals were evaluated and confirmed after liver biopsy. The exclusion criteria consisted of significant alcohol abuse (< 20 g daily), evidence of hepatitis B and C, evidence of drug-induced fatty liver disease and other specific liver diseases such as hemochromatosis, Wilson's disease or autoimmune liver disease. The patients were assessed for metabolic syndrome, and biochemical, anthropometric and histopathological evaluations were carried out. The degree of disease activity in the NAFLD patients was evaluated using the NAFLD Activity Score. The data were analyzed by SPSS, version 16.0.

RESULTS: Females predominated among the study

participants (250, 57.0%), and the mean age was 40.8 ± 10.2 years. The numbers of overweight, obese I and obese II patients were 58 (13.2%), 237 (53.9%) and 93 (21.2%), respectively. However, there were 422 (96.2%) centrally obese patients. NASH was absent in 10 (5.6%) cases, borderline in 92 (52.6%) cases and present in 75 (42.4%) cases. The presence of diabetes could significantly ($P = 0.001$) differentiate NASH from simple steatosis. The following parameters did not influence the development of NASH: age, sex, basal metabolic index, waist circumference, serum high-density lipoprotein, triglyceride, insulin resistance index, hypertension and metabolic syndrome. The serum gamma-glutamyl transpeptidase (GGT) level was significantly higher ($P = 0.05$, 51.7 ± 32.8 and 40.4 ± 22.6 U/L) in the NASH patients, with a sensitivity of 45% and a specificity of only 68%. The serum alanine aminotransferase and aspartate aminotransferase levels were not able to predict NASH.

CONCLUSION: Females were the predominant sufferers of NAFLD in Bangladesh. The prevalence of NASH was high. Diabetes was found to be the main culprit in developing NASH. GGT was the only biochemical marker of NASH. We recommend liver biopsy in NAFLD patients who have diabetes and elevated GGT.

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Key words: Fatty liver; Gamma-glutamyl transpeptidase; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Alanine aminotransferase; Obesity; Basal metabolic index

Core tip: We have designed this study to explore the prevalence of and risk factors for nonalcoholic steatohepatitis (NASH) in nonalcoholic fatty liver disease patients. Other causes of liver disease were excluded. A total of 493 patients with sonographic evidence of fatty change were considered, and 177 of these patients were evaluated and confirmed by liver biopsy after

making exclusions. Females were predominant (250, 57.0%). Central obesity was more prevalent among the patients compared with overall obesity. NASH was observed in 75 (42.4%) of the cases. The presence of diabetes and elevated gamma-glutamyl transpeptidase could differentiate NASH from simple steatosis. Serum alanine aminotransferase and aspartate aminotransferase could not be used to detect NASH.

Alam S, Noor-E-Alam SM, Chowdhury ZR, Alam M, Kabir J. Nonalcoholic steatohepatitis in nonalcoholic fatty liver disease patients of Bangladesh. *World J Hepatol* 2013; 5(5): 281-287 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v5/i5/281.htm> DOI: <http://dx.doi.org/10.4254/wjh.v5.i5.281>

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a clinico-histopathological entity with histological features that resemble alcohol-induced liver injury. By definition, this disease occurs in patients with little or no history of alcohol consumption^[1]. NAFLD is the most common liver disease in western countries, affecting 20%-30% of the general population^[2,3]. The term NAFLD includes a spectrum of histological features, including simple steatosis, steatosis with inflammation, steatosis without inflammation, ballooning degeneration and pericellular fibrosis or Mallory's hyaline (nonalcoholic steatohepatitis, NASH). Primary NASH refers to steatohepatitis that is associated with dysmetabolic syndrome, whereas secondary NASH refers to steatohepatitis that accompanies other syndromes or is caused by certain drugs (for example, amiodarone)^[1]. NASH may progress to cirrhosis in up to 20% of patients^[4]. Patients with NASH are at risk for progressive liver disease (which can progress to cirrhosis, hepatocellular carcinoma, and death from chronic liver disease), as well as cardiovascular mortality and type-2 diabetes. Reports have suggested that the prevalence of NAFLD among Asian Indians is comparable to that seen in the West, and NASH may be present in approximately 20% of these patients, with a 2- to 3-fold increased prevalence in patients with type-2 diabetes^[5]. The average age for NASH patients is 40-50 years, and that for NASH-related cirrhosis is 50-60 years. The progression of fibrosis, as detected by liver biopsy, has been observed in 43% of NASH patients, whereas 54% of patients remained unchanged, and 3% showed a histological improvement during a follow-up from 1-7 years^[6]. NASH most likely causes approximately 80% of the cases of cryptogenic (extensive evaluation excluded a recognizable etiology) cirrhosis, which accounts for 10%-20% of all cirrhosis cases and progresses to advanced fibrosis in 32%-37% of patients^[7].

In parallel with the epidemic of obesity and metabolic syndrome worldwide, the prevalence of NAFLD in Asian countries has increased rapidly, with a trend toward younger patients, over the last two decades. Childhood

NAFLD has also progressively attained clinical importance. The prevalence of NAFLD has been described at 10%-39% in the populations of various locations: North America, Japan, northern and southern Europe, South America, Australia and the Middle East^[8]. NAFLD has been associated with insulin resistance and hyperinsulinemia, even in lean subjects with normal glucose tolerance^[9]. Diabetes mellitus may be an independent predictor of advanced NAFLD, including cirrhosis and hepatocellular carcinoma^[10]. NAFLD is now recognized as the hepatic component of metabolic syndrome, which includes hyperlipidemia, glucose intolerance, obesity, and systemic hypertension. The risk and severity of NAFLD increase with the number of components of metabolic syndrome that are present^[11]. The contrasting clinical course of NASH *vs* non-NASH fatty liver (NNFL) indicates that these two conditions diverge early in the course of NAFLD, although a small number of patients most likely transition from NNFL to NASH. A progression to cirrhosis is usually preceded by longstanding histological NASH and is infrequent during NNFL. Longitudinal studies with serial biopsies have shown that approximately one-third of NASH patients develop advanced fibrosis (stage 3 or 4 fibrosis) over the course of 5-10 years from the time of the initial diagnosis^[4,12]. Although it is usually relatively slow, the progression to cirrhosis can occur in as little as 2-3 years. NASH is a common cause of "cryptogenic" cirrhosis, which accounts for 10%-20% of all cirrhosis cases^[13]. Among patients diagnosed with NASH-related cirrhosis, the risk of developing portal hypertension (a major complication) is 17%, 23% and 52% at 1, 3 and 10 years, respectively. Among patients with early-stage NASH, the overall mortality over 10-15 years is approximately 10%-12%, being significantly higher in the NASH *vs* the NNFL patients, compared to the general population. The risk of developing decompensated cirrhosis is 5%-10%, and that of hepatocellular cancer is 1%-2%. There is a ten-fold increased risk of cirrhosis relative to the general population^[14].

A complete diagnosis of fatty liver disease should ideally define the histology, the stage and grade of the disease and its etiology. In Bangladesh, NAFLD has never been sufficiently addressed by the medical community. NASH is a potentially dangerous condition that requires medical intervention. The prevalence of NASH and the potential risk factors for it have not been previously explored. We have designed this study protocol to estimate the prevalence of NASH in NAFLD patients and the risk factors for developing NASH in the context of Bangladesh; the results will contribute to future scientific knowledge and interventions.

MATERIALS AND METHODS

Study population

We initially included 439 patients from the Outpatient Department of Hepatology in the University Hospital during the period of March 2010 to December 2012 who

were referred due to fatty infiltration in the liver according to ultrasonography. The exclusion criteria consisted of significant alcohol abuse (> 20 g daily), evidence of hepatitis B and C, evidence of drug-induced fatty liver or other specific liver diseases such as hemochromatosis, Wilson's disease or autoimmune liver disease. These patients underwent clinical evaluations, anthropometric measurements, and blood tests. Liver biopsies were performed in 190 patients, but 4 of these biopsy samples were inadequate to assess for histopathology, and another 4 patients withdrew from the study. The histopathological reports of 182 patients were available, but 5 of them did not present fatty change upon microscopy. We therefore included 177 patients for further analysis. The study was approved by the Institutional Review Board, and all of the individuals provided written informed consent prior to enrollment in the study. Metabolic syndrome was defined according to Asian criteria^[15], and three of the five listed criteria were considered: waist circumference ≥ 80 cm for women and ≥ 90 cm for men, serum triglyceride ≥ 150 mg/dL (1.7 mmol/L), serum high-density lipoproteins (HDL) cholesterol < 50 mg/dL (1.3 mmol/L) for women and < 40 mg/dL (1 mmol/L) for men, elevated blood pressure (systolic blood pressure ≥ 130 and or diastolic blood pressure ≥ 85 mmHg or drug treatment for hypertension) and plasma glucose concentration ≥ 100 mg/dL (5.6 mmol/L) or drug treatment for diabetes.

Clinical and biochemical evaluation

All of the patients were clinically evaluated, and blood pressure, body mass index (BMI) and waist circumference were recorded for every patient. Liver function tests were performed prior to the liver biopsy. Blood samples were obtained under fasting conditions, and the following tests were performed using standard laboratory methods: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, gamma glutamyl transpeptidase (GGT), international normalized ratio, blood glucose (fasting and 2 h after breakfast), lipid profile, and insulin level (which was assessed using the method of indirect chemiluminescence). Insulin resistance was calculated according to the Homeostatic Model Assessment index.

Histological assessment

Liver biopsy specimens of the 177 patients were analyzed by a pathologist who was blinded to the clinical and biochemical results of the patients. The diagnosis of NASH was based on the criteria of Brunt *et al.*^[16], as modified by Kleiner *et al.*^[17]. In this scoring system, the degree of disease activity in NAFLD was evaluated using the NAFLD Activity Score (NAS), which was calculated as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and hepatocyte ballooning (0-2); therefore, the score ranged from 0 to 8. A NAS of 5 or more was diagnosed as "definitive NASH", a NAS of 2 or less as "non-NASH", and a NAS of 3 or 4 as "borderline NASH". Diagnoses other than NASH were considered

to be NNFL. The hepatic fibrosis staging was as follows: 0 = no fibrosis; 1 = zone 3 fibrosis only; 2 = zone 3 and portal/periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis.

Statistical analysis

The results are presented as the mean \pm SD for the quantitative data and as numbers or percentages for the categorical or qualitative data. The statistical differences in the quantitative data were assessed using a *t* test or one-way analysis of variance. The qualitative data were compared using the χ^2 test. For all of the tests, significance was achieved at $P < 0.05$.

RESULTS

Patient characteristics

A total of 177 patients were included in this study. There were 104 females (58.8%) and 73 males (41.2%). The mean age of the sample was 40.1 ± 9.5 years. Most of the affected individuals were aged 31 to 40 years (66, 37.3%), and the remainder were aged 41 to 50 years (59, 33.3%). The majority of the population comprised housewives (94, 53.1%), but there were also service holders (26, 14.6%), businessmen (23, 13.0%) and students (34, 19.3%). Hypertension and diabetes were present in 41 (23.1%) and 39 (22.1%) of the patients, respectively, but metabolic syndrome was present in 93 (52.3%) of the patients. Triglycerides were high in 130 (73.9%) of the patients. The BMI was normal in 24 (13.5%) of the patients, overweight in 14 (8.1%) of the patients, obese I in 88 (49.3%) of the patients and obese II in 51 (29.1%) of the patients, according to the criteria for Asians^[18]. Most of the patients presented central obesity (171, 96.5%), with a waist circumference above normal. The ALT, AST and GGT levels were 56.7 ± 35.9 , 46.6 ± 50.5 and 46.2 ± 28.6 U/L, respectively. The insulin resistance index was higher than normal in 79 (44.6%) of the patients.

Histological changes

The histopathological reports of 177 patients were available for further analysis. There was no significant difference between the biopsied and non-biopsied patients regarding the clinical, anthropometric and biochemical variables. Steatosis of < 33% was observed in 73 (41.2%) of the patients, steatosis of 33%-66% was observed in 82 (46.3%) of the patients, and steatosis of > 66% was observed in 22 (12.4%) of the patients. Lobular inflammation was absent in 10 (5.6%) of the patients, mild in 93 (52.5%) of the patients, moderate in 70 (39.5%) of the patients and severe in 4 (2.3%) of the patients. Ballooning was absent in 5 (2.8%) of the patients, there was a small amount of ballooning in 138 (78.0%) of the patients, and there was prominent ballooning in 34 (19.2%) of the patients. No fibrosis was observed in 28 (15.8%) patients, stage I was observed in 94 (53.3%) patients, stage II was observed in 40 (22.5%) patients, and stage III was observed in 15 (8.3%) patients. None of the pa-

Table 1 Clinical, anthropometric and biochemical characteristics of the non-nonalcoholic steatohepatitis fatty liver and nonalcoholic steatohepatitis patients

Variable	NNFL <i>n</i> = 102	NASH <i>n</i> = 75	<i>P</i> value
Age, yr, (mean ± SD)	39.3 ± 9.4	41.0 ± 9.7	0.24
Sex: male/female	42/60	31/44	1.00
Body mass index (kg/m ²)	27.8 ± 3.9	27.8 ± 4.6	0.998
Waist (cm)			
Male	93.0 ± 5.5	93.0 ± 9.8	0.081
Female	95.8 ± 9.9	95.6 ± 11.0	0.927
HDL (mg/dL)			
Male	36.3 ± 8.9	34.2 ± 6.5	0.337
Female	39.8 ± 10.3	39.2 ± 10.3	0.801
Serum triglyceride (mg/dL)	225.2 ± 165.8	239.8 ± 111.6	0.509
Insulin resistance index	1.8 ± 1.3	1.5 ± 0.7	0.337
Diabetes (present/absent)	13/86	25/48	0.001
Hypertension (present/absent)	17/65	17/48	0.555
Metabolic syndrome (present/absent)	41/41	39/32	0.328
ALT (U/L)	56.9 ± 38.8	56.3 ± 31.8	0.603
AST (U/L)	46.9 ± 63.7	46.1 ± 22.2	0.916
GGT (U/L)	40.4 ± 22.6	51.7 ± 32.8	0.05

NNFL: Non-nonalcoholic steatohepatitis fatty liver; NASH: Nonalcoholic steatohepatitis; HDL: High-density lipoproteins; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase.

Table 2 Multivariate regression analysis for the factors influencing the development of nonalcoholic steatohepatitis

Model	Unstandardized coefficients		Standardized coefficients Beta	<i>t</i>	Sig.
	<i>B</i>	SE			
Constant	1.247	0.517		2.411	0.018
BMI	0.014	0.018	0.124	0.780	0.438
Diabetes	0.260	0.125	0.227	2.084	0.040
Serum triglyceride	0.000	0.000	-0.105	-0.919	0.361
GGT	0.005	0.002	0.289	2.473	0.015
Waist circumference	-0.004	0.008	-0.077	-0.491	0.624

BMI: Body mass index; GGT: Gamma-glutamyl transpeptidase; Sig.: Significance.

tients presented stage IV fibrosis.

According to the NAS scoring system, NASH was absent in 10 (5.6%) cases, borderline in 92 (52.6%) cases and positive in 75 (42.4%) cases. Consequently, NNFL was present in 102 (57.6%) of the cases, and NASH was present in 75 (42.4%) of the cases.

Factors leading to NASH

The prevalence of NASH in NAFLD was 75 (42.4%). There were no significant differences of age, BMI, waist circumference, serum HDL and triglyceride level, or insulin resistance index. Sex, hypertension, and metabolic syndrome did not exert influences on the development of NASH. The mean age, BMI and waist circumference were similar in NNFL and NASH patients. The mean triglycerides were higher in the NASH cases, and the mean HDL was lower in the NASH cases, but this difference was not significant. The presence of diabetes could sig-

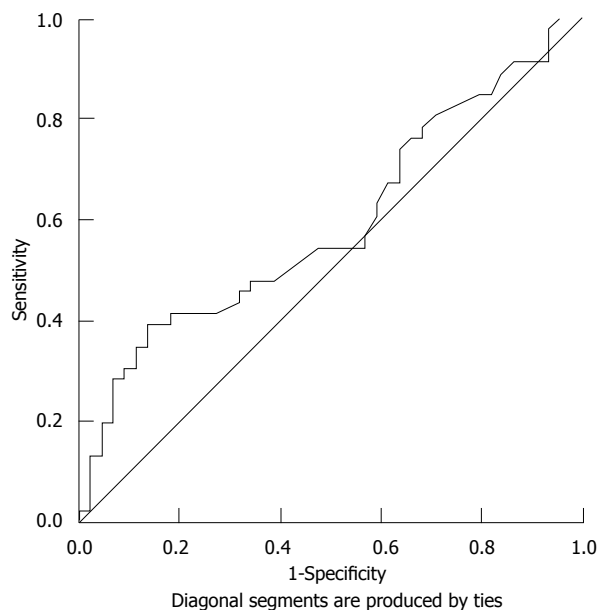


Figure 1 Receiver operating characteristic curve for gamma-glutamyl transpeptidase to differentiate nonalcoholic steatohepatitis from non-alcoholic steatohepatitis fatty liver.

nificantly (*P* = 0.001) differentiate NASH from NNFL. The serum ALT and AST levels could not be used to distinguish NASH from NAFLD. However, the serum GGT level in the NASH cases was significantly (*P* = 0.05) higher than that in the NNFL cases (Table 1). The GGT level for the NASH patients was 51.7 ± 32.8 U/L, and it was 40.4 ± 22.6 U/L for the NNFL patients. Multivariate regression analysis was also used to explore whether the presence of diabetes could influence the development of NASH (*P* = 0.04) and whether GGT could differentiate NASH from NNFL (*P* = 0.01) (Table 2). However, the area under the curve was 59.3% for GGT to differentiate NASH, with a sensitivity of 45% and a specificity of only 68% at 44.5 U/L (Figure 1).

DISCUSSION

This study is the largest series from Bangladesh in NAFLD. Reports of biopsy-confirmed NASH and NNFL are also rare. The university hospital is a tertiary care “center of excellence” hospital, and the patients are referred from all across the country. Consequently, this study may be representative of the prevalence of NASH in NAFLD throughout Bangladesh. The population-based prevalence of NAFLD had not previously been examined in Bangladesh. Most of our NAFLD patients were between 30 and 50 years of age; this result is similar to that of several reports from Asia^[6,19,20]. However, age did not influence the development of NASH. The female preponderance in NAFLD conflicts with reports from developed countries. Many recent studies have reported that males are at a higher risk for fatty liver disease^[21]. For example, in a study of 26527 subjects undergoing medical checkups, the prevalence of NAFLD was 31% in men and 16%

in women^[22]. The female preponderance (250, 57.0%) observed in our study may be the result of the socially conservative attitude that led many of the women in our study to remain at home to attend to household activities without a job, leading to a sedentary life style. A similar female preponderance was observed in a population study from India^[23]. However, in accordance with previous studies, sex did not influence the development of NASH from NAFLD.

Central obesity was observed in 171 (96.5%) of the patients, which was more common than overall obesity. The prevalence of NAFLD was higher following increases in BMI or abdominal circumference, according to a report from Japan^[24]. However, other reports concluded that waist circumference served as an independent predictor of the advanced histological changes in NAFLD, rather than BMI^[25,26]. However, waist circumference was similar between the NASH and NNFL cases in our series. This result could be explained by the fact that waist circumference indicates visceral obesity but has no influence on the pathogenesis of NASH at the stage of the 2nd hit. Hypertriglyceridemia was very common (130, 73.9%) in this study, but there was no difference between the NASH and NNFL patients. TG has long been considered to be a major factor in the development of NAFLD^[5-8], but there is mounting evidence that these non-TG lipid molecules are implicated in the pathogenesis of NASH via the process of lipotoxicity. Conversely, the formation of TG may actually be a cytoprotective mechanism in the liver^[27,28]. Our study observed that the prevalence of NASH was 42.4% (75 cases) among NAFLD patients, which is high. This rate is alarming for a country such as Bangladesh. This issue has been neither addressed previously nor considered by other studies. In a previous review, NAFLD was highly prevalent (15%-45%) in modern societies, but only 10%-25% of cases developed NASH, hepatic fibrosis leading to cirrhosis, end-stage liver disease or hepatocellular carcinoma^[29]. In other studies, the prevalence of NASH was 10%-30% in NAFLD^[30] and was less in Asian populations than in European populations^[31,32]. We were unbiased in selecting patients to undergo liver biopsy, and the choice was made irrespective of the clinical, biochemical and anthropometric status of the study population. Consequently, these results can be considered to be representative of the prevailing conditions in Bangladesh society. This finding warrants further extensive study on the prevalence of NASH in Bangladesh and indicates that the awareness of the clinician is essential to diagnose NASH and to offer advice regarding possible interventions as early as possible.

The presence of diabetes indicated the presence of NASH in our study population ($P = 0.001$). Metabolic syndrome was observed in 188 (42.9%) of the patients. NAFLD is strongly associated with insulin resistance (IR) and other components of the metabolic syndrome, such as type-2 diabetes mellitus, central obesity, hyperlipidemia, and hypertension^[33]. The pathogenesis of NASH appears to be a multi-factor process. The initial insult is

the development of macrovesicular steatosis with the accumulation of hepatic fat from decreased hepatic free fatty acid oxidation and/or increased hepatic de novo lipogenesis and/or decreased lipid export from the liver. Although IR can contribute to this dysregulation of lipid metabolism, once fatty liver develops, it can worsen hepatic IR and diabetes, contributing to a vicious cycle^[34].

Serum ALT and AST levels were similar in the NASH and NNFL patients in this study. However, GGT was significantly higher ($P = 0.05$) in the NASH cases than in the NNFL cases. NASH has been associated with a slight elevation of liver enzymes, mostly ALT^[35]. In other reports, NAFLD patients typically present with asymptomatic serum aminotransferase levels that are 2-3 times higher than normal^[36]. This difference was due to variations in the selection criteria. GGT is a sensitive indicator of liver damage^[37]. An excess deposition of fat in the liver is associated with an elevated serum GGT^[38]. Recent reports suggest that an increased GGT level is a risk factor for advanced fibrosis in NAFLD, and in combination with weight loss, a decrease in GGT activity is predictive of improved lobular inflammation and fibrosis of the liver^[39].

The limitation of this study was that it was not performed at the community level but rather at the tertiary level hospital in the country.

In conclusion, females were the predominant sufferers of NAFLD in Bangladesh. The prevalence of NASH was high in NAFLD patients. Diabetes was the main contributor to the development of NASH in NAFLD cases. GGT was the only biochemical predictor of NASH, but it suffered from a low sensitivity and specificity. We recommend liver biopsy in NAFLD patients with diabetes and increased GGT.

COMMENTS

Background

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease, and it encompasses a histological spectrum that ranges from simple steatosis to hepatic steatosis with a necroinflammatory component, as well as nonalcoholic steatohepatitis (NASH) that may progress to cirrhosis.

Research frontiers

The area of research was the prevalence of NASH and the clinical and biochemical predictors to detect NASH in fatty liver.

Innovations and breakthroughs

Transaminases are minimally elevated in NASH, but the reports are conflicting. It was previously thought that fatty liver was a consequence of obesity. However, many normal weight people are suffering from the disease, especially in Asia.

Applications

Females were the predominant sufferers of NAFLD in Bangladesh. The prevalence of NASH was high. Diabetes was mainly associated with NASH. Gamma-glutamyl transpeptidase (GGT) was the only biochemical predictor of NASH, but it showed a low sensitivity and specificity.

Peer review

The authors offer the first report on a hospital-based cohort of NAFLD patients from Bangladesh. The cohort consists of 493 patients with an echogram-based diagnosis of fatty liver. The authors provide further analysis on 177 patients who underwent liver biopsy. The authors find that more females were affected than males, which NASH was associated with diabetes, and that GGT was significantly higher in the NASH patients.

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