ORIGINAL PAPER

DOI: http://dx.doi.org/10.15403/jgld-293

Characterization of Patients with Biopsy-Proven Non-Alcoholic Fatty Liver Disease and Normal Aminotransferase Levels

Celal Ulasoglu[1], Feruze Yilmaz Enc[1], Eda Kaya[2], Yusuf Yilmaz[2,3]

1) Department of Gastroenterology, Goztepe Teaching and Research Hospital, Medeniyet University, Istanbul
2) Liver Research Unit, Institute of Gastroenterology, Marmara University, Istanbul
3) Department of Gastroenterology, School of Medicine, Marmara University, Istanbul, Turkey

Address for correspondence:
Yusuf Yilmaz
Marmara Universitesi, Gastroenteroloji Enstitusu, P.K. 53, Basibuyuk, Maltepe, 34840 Istanbul, Turkey
dryusufyilmaz@gmail.com

Received: 17.07.2019
Accepted: 22.10.2019

ABSTRACT

Background & Aims: Non-alcoholic fatty liver disease (NAFLD) is one of the major causes of abnormal liver function tests in hepatology practice. However, not all patients with NAFLD have increased aminotransferase levels. The aim of this study was to compare the clinical and histologic characteristics of patients with biopsy-proven NAFLD showing normal versus elevated aminotransferase levels.
Methods: We retrospectively reviewed 515 patients with biopsy-proven NAFLD. Patients with ALT ≤ 40 U/L and AST ≤ 37 U/L were considered as having normal liver enzymes. A histological fibrosis score F ≥ 3 was used to define advanced fibrosis.
Results: Of the 515 study participants, 107 (20.8%) had normal liver enzymes. Compared with patients showing elevated liver enzymes, those with normal aminotransferase levels were older and most commonly women. Moreover, they had a higher body mass index and more frequently showed metabolic risk factors (metabolic syndrome, diabetes mellitus, hypertension, higher waist and hip circumferences). Although liver histology tended to be less severe in patients with normal liver enzymes, the prevalence of advanced fibrosis was similar in the two groups. Diabetes mellitus (odds ratio [OR] = 2.12, 95% confidence interval [CI] = 1.46–3.91, $p < 0.001$) and age (OR = 1.14, 95% CI = 1.07–1.24, $p < 0.05$) were identified as independent predictors of advanced fibrosis in patients with normal aminotransferase levels.
Conclusions: NAFLD with normal aminotransferase levels is characterized by a severe metabolic profile and a prevalence of advanced fibrosis similar to that identified in cases with elevated aminotransferase levels.

Key words: non-alcoholic fatty liver disease – liver enzymes – aminotransferases – biopsy – advanced fibrosis

Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferases; BMI: body mass index; CI: confidence interval; CRN: clinical research network; HDL: high-density lipoprotein; HOMA-IR: homeostasis model assessment of insulin resistance; LDL: low-density lipoprotein; NAFL: non-alcoholic fatty liver; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; MS: metabolic syndrome; NAS: non-alcoholic fatty liver disease activity score; OR: odds ratio; SAF: steatosis, activity, and fibrosis.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), which comprises a complex spectrum of fatty liver changes [1], represents a growing health burden and is one of the major causes of abnormal liver function tests in hepatology practice [2, 3]. It is generally considered as the hepatic manifestation of the metabolic syndrome (MS) [4], with overweight and obesity being the predominant underlying risk factors [5]. The diagnosis of NAFLD is generally suspected in overweight/obese subjects with elevated aminotransferase levels [6].

However, it is widely recognized that increased liver enzymes are not invariably present in all patients with NAFLD. Mofrad et al. [7] have previously shown that NAFLD may show histological progression even in the presence of normal alanine aminotransferase (ALT) levels. In addition, the histologic features of patients with normal ALT were similar to those of patients with elevated serum aminotransferases [7]. Similar findings were reported by Uslusoy et al. [8] in a small study focusing on 34 patients with ultrasound-diagnosed liver steatosis. Fracanzani et al. [9] reported that normal ALT was not a reliable criterion to exclude patients with suspected NAFLD from liver biopsy. Interestingly, aminotransferase

levels within normal ranges have been closely associated with the severity of metabolic syndrome, the main risk factor for NAFLD, in a large population-based cohort [10].

Taking advantage of a large clinical cohort, we designed the current study to compare the clinical and histological characteristics of patients with biopsy-proven NAFLD showing normal versus elevated serum aminotransferase levels. Moreover, we sought to identify the independent predictors of advanced fibrosis (F ≥3), which is the main adverse prognostic determinant in NAFLD [11, 12], in patients with normal versus elevated aminotransferase levels.

METHODS

Study patients

This research was designed as a retrospective review of prospectively collected data. The study sample consisted of 515 consecutive adult patients with biopsy-proven NAFLD recruited from the outpatient facilities of the Department of Gastroenterology at Marmara University School of Medicine and the Institute of Gastroenterology, Istanbul, Turkey. Indications for liver biopsy were as follows: 1) evidence of hepatic steatosis on ultrasound and/or fibrosis on transient elastography; 2) hepatomegaly or elevated aminotransferase levels, and 3) absence of secondary causes of hepatic fat accumulation (e.g., significant alcohol consumption [>21 units of alcohol per week in men and >14 units of alcohol per week in women] and previous history of steatogenic drugs use). Patients were excluded if they met one of the following criteria: presence of viral hepatitis, drug-induced liver disease, autoimmune hepatitis, genetic liver diseases, and Wilson's disease. In all patients a complete medical history was taken and all underwent physical examination. The procedures used for data collection have been reported in detail [13-15]. Liver biopsies were processed by an experienced pathologist and a histological fibrosis score F≥3 was used to define advanced fibrosis [16]. The study followed the tenets of the Helsinki Declaration and it was approved by the local Ethics Committee. Owing to the retrospective nature of the study, the need for informed consent was waived.

Definition of normal liver enzymes

Alanine aminotransferase and aspartate aminotransferase (AST) were measured by our central clinical laboratory on an autoanalyzer. According to our hospital reference values, patients with ALT ≤40 U/L and AST ≤37 U/L were considered as having normal liver enzymes. Conversely, elevated liver enzymes were defined as ALT >40 U/L and/or AST >37 U/L.

Data analysis

Data were presented using descriptive statistics. Intergroup comparisons of normally distributed and skewed continuous variables were performed with the Student's *t*-test and the Mann-Whitney U test, respectively. The Pearson's chi-square test was used to analyze categorical variables. Independent predictors of advanced fibrosis (F≥3) on liver biopsy in patients showing normal versus elevated serum aminotransferase levels were identified by multivariate stepwise logistic regression analyses. All variables listed in Table I were entered as covariates into the multivariate models. Results are expressed as odds ratios (ORs) with their 95% confidence interval (CIs). Statistical calculations were performed using SPSS version 21.0 for Windows (IBM, Armonk, NY, USA). A value of $p < 0.05$ (two-sided) was considered statistically significant.

RESULTS

General characteristics of NAFLD patients with normal versus elevated liver enzymes

The general characteristics of the study sample are summarized in Table I. Of the 515 patients with biopsy-proven NAFLD, 107 (20.8%) had normal liver enzymes. Compared with patients showing increased aminotransferase levels, those with normal liver enzymes were older and most commonly women. Moreover, they had a higher body mass index and more frequently associated metabolic risk factors (MS, diabetes mellitus, hypertension, greater waist and hip circumferences). Finally, patients with normal liver enzymes showed lower total and direct bilirubin, hemoglobin, and creatinine levels, whereas their glucose concentrations were higher.

Liver histology in NAFLD patients with normal versus elevated liver enzymes

The results of liver histology in the 515 study patients are presented in Table II. Compared with patients with elevated liver enzymes, those with normal aminotransferase levels were found to differ with regard to the following histologic variables: grade of steatosis, stage of disease activity, stage of fibrosis (according to the steatosis, activity, and fibrosis [SAF] score), NAFLD activity score (NAS score), as well as grades of steatosis and fibrosis (according to the Kleiner classification system). Although liver histology generally tended to be less severe in patients with normal liver enzymes, the prevalence of advanced fibrosis did not show significant intergroup differences (21.5% and 18.4% in patients with normal and elevated aminotransferares, respectively, $p = 0.465$).

Independent predictors of advanced fibrosis in NAFLD patients with normal versus elevated liver enzymes

In multivariate analysis, diabetes mellitus (OR = 2.12, 95% CI = 1.46–3.91, $p < 0.001$) and age (OR = 1.14, 95% CI = 1.07–1.24, $p < 0.05$) were identified as independent predictors of advanced fibrosis in patients with normal liver enzymes. In cases with elevated liver enzymes, diabetes mellitus was the only independent predictor of advanced fibrosis (OR = 2.65, 95% CI = 1.56–3.89, $p < 0.001$).

DISCUSSION

To our knowledge, this is one of the largest reports to date focusing on the differences between patients with biopsy-proven NAFLD and normal versus elevated liver enzymes. The main results of our study can be summarized as follows: 1) normal liver enzymes can be found in approximately 21% of all patients with biopsy-proven NAFLD; 2) patients with aminotransferase levels within normal ranges were older and most commonly women, had a higher body mass index and

Table I. General characteristics of the study patients

	Patients with normal liver enzymes (ALT≤40 U/L and AST≤37 U/L)	Patients with elevated liver enzymes (ALT>40 U/L and/or AST>37 U/L)	Entire cohort	p value
Number of patients	107	408	515	-
Age, median [min-max], years	52 [29-71]	47 [18-71]	48 [18-71]	**<0.001**
Gender, men/women (n)	38/69	238/170	276/239	**<0.001**
Body mass index, median [min-max], kg/m^2	32.1 [23.3-56.0]	30.9 [18.3-51.9]	31.2 [18.3-56.0]	**0.002**
Lean/overweight/obese (n)	5/28/74	28/135/245	33/163/319	0.217
Metabolic syndrome (yes/no)	79/28	248/160	327/188	**0.013**
Type 2 diabetes mellitus (yes/no)	54/53	151/257	205/310	**0.011**
Hypertension (yes/no)	49/58	136/272	185/330	**0.017**
Hyperlipidemia (yes/no)	75/32	245/163	320/195	0.057
Waist circumference, median [min-max], cm	105 [81-147]	104 [70-146]	104 [70-147]	**0.018**
Hip circumference, median [min-max], cm	112 [92-155]	108 [63-144]	108 [63-155]	**<0.001**
AST, median [min-max], U/L	25 [11-37]	46 [20-302]	42 [11-302]	**<0.001**
ALT, median [min-max], U/L	29 [12-39]	77 [18-483]	66 [12-483]	**<0.001**
Total bilirubin, median [min-max], mg/dL	0.63 [0.21-1.82]	0.69 [0.05-6.10]	0.67 [0.05-6.10]	**0.046**
Direct bilirubin, median [min-max], mg/dL	0.13 [0.01-0.54]	0.19 [0.01-1.83]	0.17 [0.01-1.83]	**<0.001**
Total cholesterol, median [min-max], mg/dl	211 [79-417]	211 [74-419]	211 [74-419]	0.380
Triglycerides, median [min-max], mg/dL	170 [38-1107]	165 [37-716]	167 [37-1107]	0.784
HDL cholesterol, median [min-max], mg/dL	44 [26-91]	44 [18-96]	44 [18-96]	0.402
LDL cholesterol, median [min-max], mg/dL	127 [35-265]	136 [28-400]	133 [28-400]	0.136
Leucocytes, median [min-max],	7000 [2400-14700]	7100 [3200-14900]	7040 [2400-14900]	0.533
Platelets, median [min-max], × 10^3 /per microliter	225 [77-475]	241 [89-543]	239 [77-543]	0.482
Hemoglobin, median [min-max], mg/dL	13.8 [9.1-17.5]	14.7 [7.1-18.9]	14.5 [7.1-18.9]	**<0.001**
Glucose, median [min-max], mg/dL	105 [70-240]	100 [66-307]	101 [66-307]	**0.043**
Creatinine, median [min-max], mg/dL	0.71 [0.45-1.12]	0.80 [0.41-2.13]	0.77 [0.41-2.13]	**0.028**
Uric acid, mean±SD, mg/dL	5.9±1.5	6.4±1.6	6.3±1.5	0.073
Glycated hemoglobin, median [min-max], %	5.9 [4.5-8.9]	5.7 [3.52-11.1]	5.8 [3.52-11.1]	0.286
HOMA-IR, median [min-max]	4.65 [1.06-15.53]	4.71 [1.0-28.76]	4.68 [1.0-28.76]	0.282

For abbreviaitons see the Abbreviations list. Data were compared with the Student's *t*-test, the Mann-Whitney *U* test, or the chi-square test, as appropriate. Significant p values are marked in bold

Table II. Histological characteristics of the study patients

Variable	Patients with normal liver enzymes (ALT≤40 U/L and AST≤37 U/L	Patients with elevated liver enzymes (ALT>40 U/L and/or AST>37 U/L)	Entire cohort	p value
Number of patients	107	408	515	-
Length of specimen, median [min-max], mm	30 [12-46]	29 [10-65]	29 [10-65]	0.780
Number of portal tracts, median [min-max]	19 [6-24]	20 [6-41]	20 [6-41]	0.161
SAF algorithm classification, NASH / NAFL, (n)	92/15	375/33	467/48	0.091
Grade of steatosis (S) (SAF score): S0/S1/S2/S3 (n)	0/47/41/19	0/79/163/166	0/126/204/185	**<0.001**
Stage of activity (A) (SAF score): A0/A1/A2/A3/A4, (n)	3/11/36/32/25	6/23/81/136/162	9/34/117/168/187	**0.002**
Stage of fibrosis (F) (SAF score): F0/F1/F2/F3/F4, (n)	48/22/14/16/7	114/136/83/58/17	162/158/97/74/24	**0.004**
Mild disease/severe disease (n)	13/94	27/381	40/475	0.089
NAS score (NASH CRN), median [min-max]	4 [1-8]	5 [1-8]	5 [1-8]	<0.001
Significant fibrosis (F≥2), (n)	37	158	195	0.431
Advanced fibrosis (F≥3), (n)	23	75	98	0.465
Cirrhosis (F=4), (n)	7	17	24	0.305
Grade of steatosis (Kleiner): S0/S1/S2/S3 (n)	0/47/41/19	0/79/163/166	0/126/204/185	**<0.001**
Stage of fibrosis (Kleiner): F0/F1/F2/F3/F4, (n)	48/22/14/16/7	114/136/83/58/17	162/158/97/74/24	**0.004**

For abbreviaitons see the Abbreviations list. Data were compared with the Mann-Whitney *U* test or the chi-square test, as appropriate. Significant p values are marked in bold.

more frequently showed metabolic risk factors (MS, diabetes mellitus, hypertension, larger waist and hip circumferences); 3) the prevalence of advanced fibrosis did not differ significantly in patients with normal versus elevated liver enzymes, and diabetes mellitus was the main independent risk factor for advanced fibrosis in both groups.

The relatively high prevalence of biopsy-proven NAFLD without increased liver enzymes (~21%) confirms previous data indicating that aminotransferase levels alone cannot invariably reflect liver damage [7-9] – ultimately being not valuable to exclude patients from liver biopsy. This concept has been also previously referred to as "silent NASH" [17]. In a study previously conducted in 458 Italian patients with NAFLD, Fracanzani et al. [9] reported that glucose metabolism and insulin resistance in subjects with normal ALT should also be considered in the selection of NAFLD cases for histologic assessment of disease severity and progression. Here, we were able to expand these findings by showing that patients with NAFLD and normal liver enzymes can even have a higher metabolic burden than those with increased aminotransferase levels. Our results have important clinical implications moving from an era when liver biopsies were performed mainly because of persistently elevated liver enzymes irrespective of the true risk of having NAFLD. Owing to the availability of non-invasive imaging biomarkers of both steatosis and fibrosis on transient elastography (TE) [18, 19], it is recommended to perform TE in all patients with suspected NAFLD harboring metabolic risk factors (MS, diabetes mellitus, hypertension, larger waist and hip circumferences) even in the absence of elevated liver enzymes.

Although patients with normal aminotransferase levels tended to have a lower degree of histologic severity, it is noteworthy that the prevalence of advanced fibrosis – the main adverse prognostic determinant in NAFLD [12] – was similar to that observed in patients with elevated liver enzymes. The surprisingly high rate of advanced fibrosis indicates that the natural course of NAFLD with normal aminotransferases merits further scrutiny in relation to clinical outcomes and mortality. We identified two independent predictors of advanced fibrosis in NAFLD with normal liver enzymes, i.e., age and diabetes mellitus, the latter being shared with patients having elevated aminotransferases. Although diabetes is a well-known risk factor for advanced fibrosis [19, 20], our findings highlight the need to thoroughly screen for hepatic fibrosis all diabetic patients with NAFLD, even in the presence of normal liver enzymes.

Some strengths and caveats of the present study merit comment. The use of liver biopsies in all the patients and the large sample size are the main strengths of this report. The main limitation is that the process of subject selection for liver biopsy can be biased. Moreover, the study cohort consisted only of Turkish subjects and the results may not be applicable to other populations. Another issue that needs to be addressed in future studies is to identify the prognosis in patients with NAFLD and normal liver enzymes.

Our results suggest that NAFLD with normal liver enzymes might be characterized by a severe metabolic profile and a prevalence of advanced fibrosis similar to that of cases with elevated aminotransferases. Further research is required to clarify the clinical outcomes in this clinical entity and the pathophysiologic mechanisms by which liver enzymes are not increased in a subset of patients with NAFLD.

Conflicts of interest: None to declare.

Authors' contributions: C.U., Y.Y.: conception and design of the study; C.U., F.Y.E., E.K.: data acquisition; E.K., Y.Y.: statistical analysis; C.U., Y.Y. data analysis and drafting of the manuscript. All authors critically revised the manuscript and approved the final version.

REFERENCES

1. Hardy T, Oakley F, Anstee QM, Day CP. Nonalcoholic fatty liver disease: pathogenesis and disease spectrum. Annu Rev Pathol 2016;11:451-496. doi:10.1146/annurev-pathol-012615-044224
2. Perumpail BJ, Khan MA, Yoo ER, Cholankeril G, Kim D, Ahmed A. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. World J Gastroenterol 2017;23:8263-8276. doi:10.3748/wjg.v23.i47.8263
3. Younossi Z, Tacke F, Arrese M, et al. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology 2019;69:2672-2682. doi:10.1002/hep.30251
4. Kim D, Touros A, Kim WR. Nonalcoholic fatty liver disease and metabolic syndrome. Clin Liver Dis 2018;22:133-140. doi:10.1016/j.cld.2017.08.010
5. Yilmaz Y, Younossi ZM. Obesity-associated nonalcoholic fatty liver disease. Clin Liver Dis 2014;18:19-31. doi:10.1016/j.cld.2013.09.018
6. Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review. World J Hepatol 2017;9:715-732. doi:10.4254/wjh.v9.i16.715
7. Mofrad P, Contos MJ, Haque M, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology 2003;37:1286-1292. doi:10.1053/jhep.2003.50229
8. Uslusoy HS, Nak SG, Gülten M, Biyikli Z. Non-alcoholic steatohepatitis with normal aminotransferase values. World J Gastroenterol 2009;15:1863-1868. doi:10.3748/wjg.15.1863
9. Fracanzani AL, Valenti L, Bugianesi E, et al. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. Hepatology 2008;48:792-798. doi:10.1002/hep.22429
10. Kälsch J, Bechmann LP, Heider D, et al. Normal liver enzymes are correlated with severity of metabolic syndrome in a large population based cohort. Sci Rep 2015;5:13058. doi:10.1038/srep13058
11. Angulo P, Machado MV, Diehl AM. Fibrosis in nonalcoholic fatty liver disease: mechanisms and clinical implications. Semin Liver Dis 2015;35:132-145. doi:10.1055/s-0035-1550065
12. Ekstedt M, Hagström H, Nasr P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 2015;61:1547-1554. doi:10.1002/hep.27368
13. Yilmaz Y, Eren F. Serum biomarkers of fibrosis and extracellular matrix remodeling in patients with nonalcoholic fatty liver disease: association with liver histology. Eur J Gastroenterol Hepatol 2019;31:43-46. doi:10.1097/MEG.0000000000001240
14. Sunbul M, Agirbasli M, Durmus E, et al. Arterial stiffness in patients with non-alcoholic fatty liver disease is related to fibrosis stage and epicardial adipose tissue thickness. Atherosclerosis 2014;237:490-493. doi:10.1016/j.atherosclerosis.2014.10.004

15. Yilmaz Y, Ayyildiz T, Akin H, et al. Gallstone disease does not predict liver histology in nonalcoholic fatty liver disease. Gut Liver 2014;8:313-317. doi:10.5009/gnl.2014.8.3.313
16. Bilgin BO, Sunbul M, Kani HT, et al. Arterial stiffness is associated independently with liver stiffness in biopsy-proven nonalcoholic fatty liver disease: a transient elastography study. Eur J Gastroenterol Hepatol 2019 Jun 6. doi:10.1097/MEG.0000000000001471
17. Sorrentino P, Tarantino G, Conca P, et al. Silent non-alcoholic fatty liver disease-a clinical-histological study. J Hepatol 2004;41:751-757. doi:10.1016/j.jhep.2004.07.010
18. Eddowes PJ, Sasso M, Allison M, et al. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology 2019;156:1717-1730. doi:10.1053/j.gastro.2019.01.042
19. Demir M, Deyneli O, Yılmaz Y. Screening for hepatic fibrosis and steatosis in Turkish patients with type 2 diabetes mellitus: A transient elastography study. Turk J Gastroenterol 2019;30:266-270. doi:10.5152/tjg.2018.18559
20. Patel P, Hossain F, Horsfall LU, et al. A pragmatic approach identifies a high rate of nonalcoholic fatty liver disease with advanced fibrosis in diabetes clinics and at-risk populations in primary care. Hepatol Commun 2018;2:893-905. doi:10.1002/hep4.1208