Research Article

Natural history of NAFLD

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The natural course of non-alcoholic fatty liver disease

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Abstract

Background and Aim: The present study aims to describe the characteristics and long-term clinical outcomes of patients with non-alcoholic fatty liver disease (NAFLD).

Material and Methods: A total of 1308 individuals with NAFLD were seen in the Liver Diseases Outpatient Clinic. Diagnosis of NAFLD in each case was based on biochemical, radiological and histological criteria, when available. After diagnosis, all NAFLD patients were administered a conventional diet and exercise program. The median follow-up period was 55.3 months.

Results: At the time of the diagnosis, the mean age was 50.8±11.3 years, and female gender was slightly predominant (51.4%). The median body mass index was 29.2±4.7 kg/m^2: 39% were obese. Seventeen percent of the patients had diabetes mellitus, 53% insulin resistance, 60% hyperlipidemia, and 32% hypertension. Median serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase levels were 31 U/L (range: 10–248 U/L), 45 U/L (range: 10–285 U/L) and 41 (range: 8–1200 U/L), respectively. Liver biopsy was performed in 293 individuals. The median NAFLD activity score was 5.0, median hepatic steatosis 2, ballooning 1, lobular inflammation 1, portal inflammation 0, and fibrosis 0. Of note, 41.3% of the samples (121/293) revealed the presence of fibrosis and 31% of the samples (37/121) showed significant fibrosis. With multivariate analysis, diabetes and obesity were associated with the presence of significant fibrosis. Among them, 765 patients (M/F: 353/412, mean age: 51.0±10.9) had at least six months of follow-up. In this group, from baseline to the end of the follow-up period, a significant improvement in the serum AST and ALT levels was observed.

Conclusion: NAFLD is a potentially progressive disease. Diabetes and obesity were associated with the presence of advanced fibrosis.

Keywords: Cirrhosis; non-alcoholic fatty liver disease; steatohepatitis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently one of the most common causes of chronic liver disease.[1–4] NAFLD has become a major health problem affecting 2.8% to 46% of the adult population in western countries, depending on the definition used.[3–6] NAFLD is also a growing public health problem in Turkey.[6]

NAFLD is associated with obesity, insulin resistance (IR), type 2 diabetes mellitus, hypertension, hyperlipidemia, and cardiovascular disease.[1–3,7–10] NAFLD comprises a wide spectrum of liver injury, ranging from pure steatosis to non-alcoholic steatohepatitis (NASH) and to end-stage liver disease.[1–3,11–14] NASH is an aggressive clinical spectrum of the disease and can lead to cirrhosis and its complications and hepatocellular carcinoma (HCC).[1–3,11–14]

Understanding the natural history of NAFLD is important because it can guide clinicians in deciding which NAFLD patients require advanced evaluation and management and a strict follow-up period. Investigators have been working to elucidate the pathogenesis and natural course of NAFLD and the mechanism for its progression to cirrhosis.[2,15–20] However, the natural history of NAFLD and the risk factors for the progression of the disease remain uncertain. The present study aims to describe the characteristics and long-term clinical outcomes of the NAFLD patients and to identify the predictors for progression to advanced fibrosis.

Materials and Methods

This was a retrospective longitudinal study. A total of 1308 individuals diagnosed with NAFLD were seen in the liver diseases outpatient clinic between January 2001 and May 2009 were included in this study. Data were obtained from patient visit charts. Diagnosis of NAFLD in each case was based on biochemical, radiological and histological criteria, when available and on the exclusion of other forms of acute and chronic liver diseases.[1,2,11,21] Cirrhosis was defined clinically (thrombocytopenia, increased prothrombin time, hypoalbuminemia and signs of decompensation).

Methods

Liver Injury and Function Tests

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, bilirubin, fasting glucose, cholesterol, triglycerides levels, and complete blood cell counts were measured by our central laboratory. For exclusion of other forms of liver disease, serological markers for viral infections (anti-HAV immunoglobulin G [IgM], HBsAg, anti-HBs, HBeAg, anti-HBe, antiHBc IgM, IgG, anti-HCV, anti-cytomegalovirus, anti-herpes simplex virus, anti-Epstein-Barr virus, serum iron, ferritin, copper, ceruloplasmin, and alpha-1 antitrypsin levels were measured, and serological studies for anti-nuclear antibody, anti-smooth muscle antibody and anti-mitochondrial antibodies were performed. All pa-

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tients underwent abdominal ultrasonography, confirming the presence of fatty infiltration of the liver. Laboratory tests were performed at the time of diagnosis and also during the follow-up period, and during liver enzyme flare-ups.

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Obesity was defined based on World Health Organization criteria with the BMI of 25-29.9 kg/m^2 defining overweight and BMI ≥30 kg/m^2 defining obesity.[22] IR was calculated on the basis of fasting plasma glucose and insulin values using the homeostasis model assessment-IR method (HOMA-IR: plasma glucose (mg/dL) x insulin (μu/mL)/405).[23]

Histological Assessments

All liver biopsy specimens were retrieved from the archives of the Department of Pathology. The liver biopsy specimens were evaluated by one pathologist (BS) blinded to the clinical and biochemical data. Histological features of the samples were interpreted based on the criteria of Kleiner et al.[24] Fibrosis was scored from 0 to 4, and significant fibrosis was defined as stage 2 to 4 fibrosis.

Diet and Exercise

After diagnosis of NAFLD was confirmed, the management was focused in the following areas: establishment of an appropriate diet, including a conventional diet of 25 kcal/kg x ideal body weight (kg) with three meals per day containing 60% carbohydrate, 25% fat and 15% protein and an exercise program, including walking (initially as 300 steps per day for three days, thereafter adding 500 steps at 3-day intervals until a level of 10000 steps was attained) and/or jogging (20 minutes twice a day)[25,26] improvement in associated conditions; and discontinuation of potentially hepatotoxic drugs such as herbal medicine. All subjects were interviewed by the dietitian, and the same diet and exercise program was suggested.

Follow-up

All subjects were seen in the fourth week and in three or six-month intervals thereafter in the outpatient clinic.

Statistical Analyses

Nominal variables were evaluated by chi-square test. Changes within groups concerning continuous measurements were tested by a random-effects model. A comparison among groups for continuous variables was assessed by One-way ANOVA. When the p-value from the One-way ANOVA test statistics was statistically significant, Tukey's HSD test was used to determine which groups differed from which others. Wilcoxon signed ranks test was used to analyze the difference between two related samples. To define risk factors of the outcome variable (fibrosis), univariate and multiple logistic regression analyses were used. Crude and adjusted odds ratios and their 95% confidence intervals were calculated. For all tests, a two-tailed p value of less than 0.05 was considered statistically significant. Analyses were carried out with SPSS for Windows 11.5 and SAS for Windows V8.

Results

A total of 1308 patients were diagnosed with NAFLD. The present cohort consisted of 1284 individuals with NAFLD. The median follow-up period was 55.3 months (range: 6-107 months). At the time of the diagnosis, the mean age was 50.8±11.3 years, and female gender was slightly predominant (M/F: 624/660, 51.4%). The median BMI was 29.2±4.7 kg/m^2: 47% of the patients were overweight, and 39% were obese. Seventeen percent of the patients had diabetes mellitus, 53% IR (HOMA score >2.7), 60% hyperlipidemia, and 32% hypertension. Cirrhosis was clinically diagnosed in three patients. The characteristics of all patients with NAFLD are shown in Table 1.

At the time of the diagnosis, median serum AST, ALT and GGT levels were 31 U/L (range: 10-248 U/L), 45 U/L (range: 10-285 U/L) and 41 (range: 8-1200 U/L), respectively (Table 1); 39% of the NAFLD patients had normal serum ALT levels based on our reference standard (n<37 U/L).

Among them, 765 patients (M/F: 353/412, mean age: 51.0±10.9) had at least six months of follow-up. In this group, from baseline to the end of the follow-up period, a significant improvement in the serum AST and ALT levels was observed (Fig. 1a, b). The mean baseline value for serum AST level was 39.3±27.4 U/L and for serum ALT was 57.6±41.4 U/L; These values were 31.0±19.5 U/L and 42.3±31.1 U/L at the 12 months (n=694, p<0.001), 30.2±14.0 U/L and 42.5±27.6 U/L at 24 months (n=286, p<0.001), 29.8±14.2 U/L and 40.3±25.1 U/L at 36 months (n=237, p<0.001), 33.9±33.6 U/L and 39.6±25.1 U/L at 48 months (n=147, p<0.01), 31.0±18.2 U/L and 40.4±29.7 U/L at 60 months (n=104, p<0.01), 35.3±36.9 U/L and 41.2±31.5 U/L at 72 months (n=69, for ALT: p=0.003), and 31.1±33.9 U/L and 34.2±23.5 U/L at 84 months (n=47, for ALT: p<0.001), respectively. BMI was unchanged (from 29.3±4.7 kg/m^2 to 28.8±4.3 kg/m^2, p>0.05) (Fig. 1c). The improvement in ALT levels was more significant in patients with high baseline serum ALT levels than in patients with normal baseline serum ALT levels (mean ALT from 76.8±40.6 U/L to 48.6±31.0 U/L vs 23.9±7.5 U/L to 25.3±10.5 U/L, p<0.001, respectively).

Liver Histology

At the time of the diagnosis, liver biopsy was performed in 293 individuals, primarily due to abnormal liver injury tests. The median NAFLD activity score was 5.0 (range: 1 to 8), median hepatic steatosis 2 (0-3), ballooning 1 (1-3), lobular inflammation 1 (0-3), portal inflammation 0 (0-2), and fibrosis 0 (0-3) (Table 2a). Of note, 41.3% of the samples (121/293) revealed the presence of fibrosis, and 31% of the samples (37/121) showed significant fibrosis. Interestingly, 12% of patients (14/121) with biopsy-proven fibrosis had normal serum ALT levels. The histological features are shown in Table 2a. In logistic regression analysis, only diabetes mellitus (p=0.003) and obesity (p=0.021) were associated with the presence of significant fibrosis (Table 2b).

Follow-up

No patient had developed signs of advanced liver disease, none require liver transplantation, and there was no liver-related death recorded during the follow-up period.

Discussion

This study aimed to describe the long-term natural course of NAFLD in a tertiary single center. This study represents the natural history of NAFLD more accurately than any of the previous reports because each case was accurately diagnosed by specialists in hepatology based on biochemical, radiological and histological criteria, when available.

Table 1. Characteristics of all individuals with non-alcoholic fatty liver disease

	1284 NAFLD patients Mean±SD (median)	765 patients with a minimum of six months follow-up Mean±SD (median)
Age (year)	50.8±11.3 (52)	51.0±10.9 (52)
Gender (M/F)	624/660	353/412
BMI (kg/m^2)	29.2±4.7 (28.4)	29.3±4.7 (28.5)
Obesity	487 (38.9%)	306 (40.9%)
Diabetes (%)	224 (17.4%)	141 (18.4%)
HOMA-IR (n=445)	2.9	3.0
IR (% >2.7) (n=445)	237 (53.3%)	182 (40.9%)
Hypertension (%)	405 (31.5%)	249 (32.5%)
Hyperlipidemia (%)	775 (60.4%)	479 (62.6%)
Fasting glucose (n=75–115 mg/dL)	106.5±33.5 (98)	106.0±32.7 (98)
Cholesterol (n=120–200 mg/dl)	206.5±47.5 (206)	205.4±45.6 (206)
Triglyceride (n=40–200 mg/dl)	185.3±108.4 (160)	181.5±100.2 (157)
Serum ALT level (n=10–37 U/L)	56.3±42.9 (45)	57.6±41.4 (48)
Serum AST level (n=10–37 U/L)	38.3±27.1 (31)	39.3±27.4 (32)
Serum GGT level (n=0–55 U/L)	60.4±73.4 (41)	62.4±82.0 (43)
Albumin (n=3.5–5.2 g/dL)	4.4±1.4 (4.2)	4.4±1.7 (4.3)
Total bilirubin (n=0.2–1.3 mg/dL)	0.9±2.7 (0.7)	0.93±3.2 (0.7)
Prothrombin time (n=10.5–14 second)	12.3±11.3 (11.8)	12.3±14.5 (11.6)
Platelet counts (n=202–386x10^3/µL)	266.4±66.7 (261)	266.4±67.2 (261)

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; GGT: Gamma glutamyl transpeptidase; HOMA-IR: Homeostasis model assessment-insulin resistance; SD: Standard deviation.

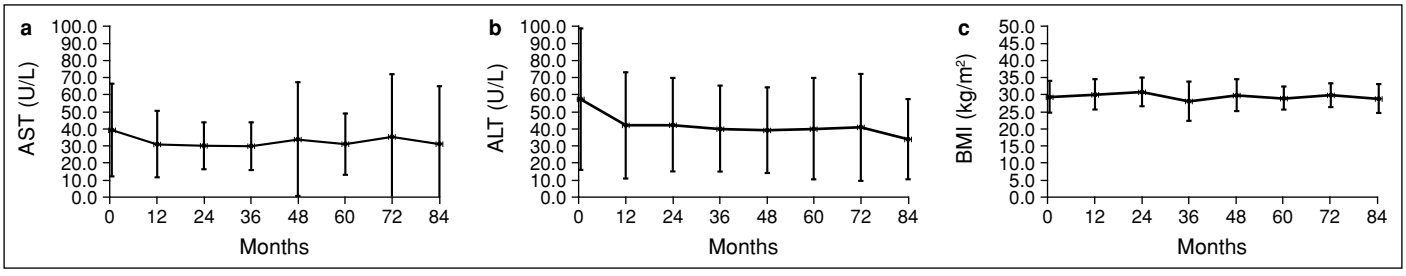

Figure 1. Alteration in biochemical (**a**: Serum AST levels, **b**: Serum ALT levels) and metabolic parameters (**c**: Body mass index) during the course of the study. Means±Standard deviation are given.

Table 2 a. Baseline histological features of the 293 patients

	Median (range)
Steatosis	2 (0–3)
0, Minimal (<5%)	n=35
1, Mild (5–33%)	n=51
2, Moderate (33–66%)	n=86
3, Severe (>66%)	n=121
Ballooning	1 (1–3)
Lobular inflammation	1 (0–3)
Portal inflammation	0 (0–2)
Fibrosis	0 (0–3)
NAS	5 (1–8)

NAS: NAFLD activity score.

NAFLD patients showed slight female predominance and were obese (39%), diabetic (17%), insulin resistant (53%), hyperlipidemic (60%), hypertensive (32%). The present data suggest that NAFLD is a burgeoning health problem and commonly coexists with metabolic disorders.

Serum AST and ALT levels have long been used as surrogate markers of liver tissue injury.[13,27–29] Serum aminotransferases levels do not correlate well with the severity of the injury[28] because several epidemiological and analytical factors are effective on serum ALT activity.[30–32] The new upper limit of normal for serum ALT activity, for men <30 U/L and for women <19 U/L, has been suggested recently to be diagnostically useful in chronic hepatitis C and NAFLD patients.[33] In a large epidemiological study, Ruhl and Everhart[34] reported that approximately 3% of the adult population had abnormal ALT levels (>43 U/L). This result increased to approximately 13% when applying the new serum ALT levels (>19 U/L).[34] In the present study, 785 NAFLD patients had abnormal serum ALT levels based on our reference value (>37 U/L),

Table 2 b. Factors associated with the presence of significant liver fibrosis

	Univariate logistic model			Multivariate logistic model		
	OR	95.0%CI (lower–upper)	p	Adj OR	95% CI (lower–upper)	p
Age >50	1.9	0.9–3.8	0.085	1.3	0.6–2.9	0.498
Female gender	2.7	1.3–5.5	0.007	1.8	0.8–3.9	0.152
Diabetes	4.8	2.3–10.1	<0.001	3.3	1.5–7.2	0.003
Hypertension	3.3	1.6–6.7	0.001			
Hyperlipidemia	0.6	0.3–1.1	0.098			
Obesity	3.4	1.6–7.1	0.002	2.5	1.1–5.6	0.021
Insulin resistance	2.0	0.8–5.4	0.154			
High serum ALT (>37 U/L)	2.6	0.8–8.9	0.123			

OR: Odd ratios; CI: Confidance interval; ALT: alanine aminotransferase.

whereas this number reached 1037 when the new ALT levels were applied. When comparing patients according to the new activity versus our reference values of ALT activity, the prevalence of obese patients and patients with abnormal ALT levels, and hyperlipidemia were significantly increased. This observation confirms the previous reports[35] that the numbers are increased with the application of the new ALT activity, but its diagnostic utility remains poor. Moreover, reducing the upper limit of serum ALT activity according to the new ALT guidelines is not useful in identifying the entire spectrum of NAFLD, except for the change in the numbers.

Several possible risk factors for progression to advanced fibrosis,[36,37] including advanced age, female gender, increased BMI, presence of diabetes, metabolic syndrome and high initial aminotransferases levels, were analyzed. However, the risk factors for progression of the advanced fibrosis remain unclear. A meta-analysis study recently reviewed risk factors for fibrosis progression in NASH in 10 selected studies comprising 221 patients with NASH.[37] The investigators demonstrated that age and inflammation on initial biopsies were independent predictive factors of progression to advanced fibrosis on subsequent liver biopsies.[37] In contrast, some investigators found that the presence of necroinflammation on the initial biopsy was not associated with progression in fibrosis during the follow-up.[38] The present study revealed that obesity and the presence of diabetes were significantly associated with advanced fibrosis on initial biopsies.

Progression to cirrhosis and HCC in some series was well documented.[18,20,38,39] Hui et al.[39] followed 23 patients with NASH-associated cirrhosis for a median of 60 months. The investigators reported that 39% of the patients had developed liver-related complications, including ascites, variceal bleeding, and encephalopathy, and 22% of the patients died due to liver failure.[39] Adams et al.[18] reported that 62% of the patients with NASH-associated cirrhosis had developed complications, and 33% of them died due to liver-related complications during a median follow-up of 82 months. In a pediatric population, Feldstein et al.[20] recently reported that among 66 children with NAFLD, two NASH-associated cirrhotic children underwent liver transplantation for decompensation, and two children with NASH died with a mean of 77 months' follow-up. In the present study, no patient with NASH-associated cirrhosis at diagnosis showed any signs of advanced liver disease during a median follow-up of 60 months, none required liver transplantation and there was no liver-related death. This difference in the progression of disease between the current and previous studies may be attributed to the presented small numbers of cirrhotics (n=3) at diagnosis. The present data suggest that NAFLD is a disease with progressive potential in adult.

Conclusion

In conclusion, NAFLD is one of the most common causes of chronic liver disease in Liver Disease Outpatient Clinics. Diabetes and obesity were associated with the presence of advanced fibrosis.

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Conflict of Interest: The authors have no conflict of interest to declare.

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