Original Article

Association between sonographic diagnosis of fatty liver with histopathologic abnormalities and liver biopsy findings in middle age patient with non-alcoholic fatty liver disease

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Abstract

Background: Liver biopsy is required to diagnose non-alcoholic steatohepatitis in patients with suspected non-alcoholic fatty liver disease (NAFLD). This study aimed to examine the relationship between sonographic diagnosis of fatty liver with histopathologic abnormalities and liver biopsy findings in patient with NAFLD.

Materials and Methods: In this cross-sectional study, a total of 180 patients, with an age range of 18-60 year old, with NAFLD based on ultrasonograghic findings were evaluated. Age, sex, body mass index, diabetes mellitus, hypertension, family history of liver disease and laboratory parameters recorded for all patients. Hence, grade of steatosis and stage of fibrosis were evaluated by liver biopsy.

Results: A total of 220 patients were enrolled. Liver biopsy was performed in 180 patients. Mean age was 43 ± 10.6 years old and 66% were male. Ultrasonograghic findings showed mild, moderate and severe NAFLD was define in 100 (55.5%), 72 (40%) and 8 (4.5%) of patients, respectively. Liver biopsies showed that steatosis scores of <5%, 5-33% and 33-66% was define in 56 (31%), 116 (64%) and 9 (5%) of patients, respectively. Furthermore, fibrosis was defined as follow; none 92 (51%), mild 68 (38%), moderate 11 (6%), bridging 5 (3%) and cirrhosis 3 (2%) patients. There was no statistically significant relationship between ultrasonograghic findings and steatosis scores ($P = 0.44$), but statistically significant relationship was found between ultrasonograghic findings and fibrosis stage ($P = 0.017$).

Conclusion: Findings revealed that, in patients with NAFLD, ultrasonographic finding were not in associate to steatosis, but were in relation with fibrosis stage.

Key Words: Fibrosis, liver biopsy, non-alcoholic fatty liver, non-alcoholic steatohepatitis, ultrasound

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Received: 12.04.2013, **Accepted:** 27.10.2013

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.186985

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is named when hepatosteatosis is present in the absence of excessive alcohol consumption.[1] It is a spectrum of liver disease that encompasses simple fatty liver, non-alcoholic steatohepatitis (NASH), advanced fibrosis and cirrhosis and is one of the most common

How to cite this article: Kalantari H, Moradi F, Hassanzade A. Association between sonographic diagnosis of fatty liver with histopathologic abnormalities and liver biopsy findings in middle age patient with non-alcoholic fatty liver disease. Adv Biomed Res 2016;5:122.

forms of chronic liver disease affecting approximately a third of adults in the United States.[2-4] In industrialized Western countries, NAFLD now affects about 20-30% of the general population.[5,6]

NAFLD can lead to hepatocellular carcinoma that is associated with several cardiovascular risk factors.[7,8] Furthermore, NAFLD is strongly related with risk factors for atherosclerosis such as hypertension, obesity, type 2 diabetes mellitus, dyslipidemia and insulin resistance.[9] Whereas, the prevalence of significant risks factor for NAFLD, such as type 2 diabetes and obesity, are increasing. Recently, as a hepatic component of metabolic syndrome, NAFLD has been accepted.[10,11] The rate of mortality in patients with NAFLD is significantly increased compared with the general population[12] and undiagnosed NAFLD may progress silently and consequences in cirrhosis, portal hypertension and liver-related death in early adulthood.[10] It is likely that increase in NAFLD will be a marked with important consequences for health care providers and is rapidly becoming an important public health problem.[10] Therefore, a practical means for the prevention of condition-associated hepatocellular damage may offer after the early detection of NAFLD by screening followed and appropriate intervention.[13]

To confirm a diagnosis of NAFLD there is no single biochemical marker[14] and a set of clinical signs and symptoms, laboratory tests, imaging tests as non-invasive markers for NAFLD presently are available. Although, they lack the specificity and sensitivity to differentiate NAFLD from NASH and, in general, a number of these signs are useful for the diagnostic evaluation of a patient with suspected NAFLD.[15] Either in patients with normal alanine aminotransferase (ALT) values the entire histological spectrum of NAFLD can be observed. And it is reported that liver enzyme levels are not sensitive for the diagnosis of NAFLD.[16,17] Despite recent advance in non-invasive diagnostic methods, a liver biopsy is still required to determine the severity of NAFLD, NASH and the presence and stage of fibrosis.[18]

Currently, the most common non-invasive method for screening asymptomatic patients with elevated liver enzymes and suspected NAFLD is ultrasonography,[19] which is easily performed and has a low cost with some limitations.[20] However ultrasonography has unsuccessful to prove efficient for the detection of inflammation and fibrosis and, it cannot be used to detect NASH and hepatic fibrosis.[15]

The risk factors and their correlation with NAFLD if can be correctly identified, they can be used in a non-invasive predictive model to evaluation the degree of fat accumulation in NAFLD. Therefore, this study was designed to evaluate the association between the clinical and laboratory markers and NAFLD, also relationship between both ultrasonography findings as a non-invasive method with liver biopsy findings as an invasive method for assessing and staging patients with NAFLD has been discussed.

MATERIALS AND METHODS

This cross-sectional study was conducted between December 2011 and October 2012, on 220 patients with NAFLD based on ultrasonograghic findings who referred to Clinics of Gastroenterology in Isfahan, Iran. The diagnosis of NAFLD was made by hepatologist on the basis of liver imaging or biopsy compatible with fatty liver; the clinical presence of ≥1 feature of metabolic syndrome with or without abnormal liver enzymes. Patients between 18 and 60 years old who undergo right upper quadrant ultrasound in the same location by an expert radiologist were eligible if they had no history of chronic liver disease, no positive test for human immunodeficiency virus, no medication associated with fatty liver (e.g. steroid, tomoxifen) and who had no history of alcohol consumption. Furthermore, patients were excluded if they had a history of total parenteral nutrition, biliopancreatic diversion, or bariatric surgery; short bowel syndrome; suspected or confirmed hepatocellular carcinoma; or unwilling to participate and refused informed consent. This study has been reviewed and approved in Isfahan University of Medical Sciences and written informed consent was obtained from all participants.

The presence grading of fatty infiltration of the liver were recorded as follow:[16]

- Mild, echogenicity was slightly increased, with normal visualization of the diaphragm and the intrahepatic vessel borders
- Moderate, was established when echogenicity was moderately increased, with slightly impaired visualization of the diaphragm or intrahepatic vessels
- Severe, echogenicity was markedly increased with poor or visualization of the diaphragm, the intrahepatic vessels and the posterior portion of the right lobe.

Data collection included age, sex, body mass index (BMI), type 2 diabetes and hypertension, family history of liver disease and laboratory parameters, which recorded for all patients. Laboratory parameters were measured from patient's blood samples as follow: Triglyceride, high-density lipoprotein (HDL)-cholesterol, cholesterol, low-density lipoprotein (LDL)-cholesterol, aspartate aminotransferase (AST), ALT and alkaline

phosphatase. BMI was calculated from weight and height (kg/m^2), hypertriglyceridemia defined as a level above the 95th percentile for age and sex;[17] low HDL-cholesterol means a level below the 5th percentile for age and sex; hypercholesterolemia means a level ≥200 mg/dL; and high LDL-cholesterol means a level ≥130 mg/dL.[18]

Patients were undergoing percutaneous liver biopsy for clinical purposes at the Clinic of Gastroenterology in AL Zahra Hospital in Isfahan, Iran. All liver biopsies were performed by radiologists under ultrasound guidance using an 18 gauge automated biopsy gun with a 1.4 mm-diameter needle and liver fragments of at least 1.5 cm in length, including eight portal tracts were considered valuable for histological assessment, then samples are sent to the histopathology lab. Liver biopsy features including grade of steatosis (0-3) and stage of fibrosis (0-4) were graded according to the scoring system proposed by Kleiner *et al*.[7] All biopsy specimens were analyzed by an expert pathologist blinded to the patient's clinical results.

Sample size was calculated using the comparison of proportions formula with two-sided log-rank test, α = 0.05 and 80% power. Data are presented as means ± one standard deviation, median interquartile range or number (%). Chi-square test for discrete variables and one way analysis of variance for continuous variables were used to compare the variables among subjects in regard to fatty liver, grade of steatosis and stage of fibrosis. Analyses were performed using Statistical Package for the Social Sciences statistical software (version 20) and two-sided *P* values were used and were considered to be statistically significant if $P < 0.05$.

RESULTS

There were 220 subjects with NAFLD based on ultrasonograghic findings and suitable for histologic evaluation. Of those, 40 subjects who unwilling to enter to the study and refused informed consent were exclude. Exclude patients were similar to other participates for age and sex. Finally liver biopsy samples from 180 subjects with NAFLD were included in this study and analyzed. Subjects included 119 (66%) males and 61 (34%) females. Mean age was 43 ± 10.6 years old. The main demographic, clinical and laboratory features are summarized in Table 1. Hypertension and type 2 diabetes were observed in 22.2% (42 patients) and 10% (18 patients) respectively.

Based on ultrasonograghic findings, mild, moderate and severe NAFLD was define in 100 (55.5%), 72 (40%) and 8 (4.5%) subjects, respectively. Data from liver biopsies showed that steatosis scores of <5%, 5-33% and 33-66% was define in 56, 116 and 9 subjects, respectively and no subjects was define with steatosis scores of >66%. Furthermore, fibrosis in studied subjects was defined as follow; none 92, mild 68, moderate 11, bridging 5 and cirrhosis 3 subject. There was no statistically significant difference between ultrasonograghic findings and steatosis scores, but statistically significant difference was found between ultrasonograghic findings and fibrosis stage [Table 2].

Table 3 provides a summary of the comparison of variables between subjects in regard to NAFLD stage based on ultrasonograghic findings. As shown, subjects with definite severe NAFLD were significantly more likely to be female and have diabetes with higher level of BMI and more frequencies of high LDL-cholesterol compare to mild and moderate NAFLD.

Comparison of demographic, lipid profile, liver enzymes between subjects in regard to steatosis grade and fibrosis stage based on biopsy findings are shown in Tables 4 and 5, respectively. A frequency of hypertriglyceridemia and high LDL-cholesterol in patients with Grade 2 steatosis were significantly lower than other steatosis grades [Table 4]. Also, a frequency of high LDL-cholesterol in patients with bridging or cirrhosis was significantly lower than other fibrosis stage [Table 5].

Table 1: Demographic, clinical and laboratory features in studied patients

Variables	
Age (year)	43±10.6
Gender	
Male	119 (66.1)
Female	61 (33.9)
Body mass index (kg/m^2)	28.3±3.3
History of type 2 diabetes	18 (10)
History of hypertension	40 (22.2)
Family history of liver disease	13 (7.2)
Hypertriglyceridemia	119 (66.1)
Low HDL-cholesterol	22 (12.2)
Hypercholesterolemia	75 (41.6)
High LDL-cholesterol	52 (28.9)
AST	43.5±44.2
ALT	79.9±26.5
Alkaline phosphatase	242.7±107.9

Data are mean±SD and number (percent). Hypertriglyceridemia defined as a level above the 95th percentile for age and sex, low HDL-cholesterol means a level below the 5th percentile for age and sex, hypercholesterolemia means a level ≥200 mg/dL and high LDL-cholesterol means a level ≥130 mg/dL. HDL: High-density lipoprotein, LDL: Low-density lipoprotein, ALT: Alanine aminotransferase, AST: Serum aspartate aminotransferase, SD: Standard deviation

Table 2: Association between ultrasonograghic findings with steatosis scores and fibrosis stage

Histologic grades	Ultrasonograghic grades			*P* value
	Mild (*n*=100)	Moderate (*n*=72)	Severe (*n*=8)	
Steatosis (%)				
Grade 0 (<5)	34 (34)	22 (30.5)	0	0.44
Grade 1 (5-32)	62 (64)	45 (62.5)	8 (100)	
Grade 2 (33-66)	4 (4)	5 (7)	0	
Grade 3 (>66)	0	0	0	
Fibrosis				
Stage 0 (none)	66 (66)	25 (34.7)	1 (12.5)	0.017
Stage 1 (mild)	22 (22)	43 (59.7)	3 (37.5)	
Stage 2 (moderate)	8 (8)	2 (2.8)	2 (25)	
Stage 3 (bridging)	4 (4)	0	1 (12.5)	
Stage 4 (cirrhosis)	0	2 (2.8)	1 (12.5)	

Data are number (percent). *P* values are resulted from Chi-square test

Table 3: Comparison of studied variables between subjects in regard to NAFLD stage based on ultrasonograghic findings

Variables	Ultrasonograghic grades			*P* value
	Mild	Moderate	Severe	
Age	41.3±10.3	44.8±11.1	49.4±14.2	0.09
Male/female (%)	64.1/35.9	73.9/26.1	20/80	0.041
Body mass index (kg/m^2)	27.6±3.4	28.8±2.6	31.1±3.9	0.033
Type 2 diabetes	4 (22.2)	7 (38.9)	7 (38.9)	<0.0001
Hypertension	16 (40)	21 (52.5)	3 (7.5)	0.08
Family history of liver	5 (38.4)	8 (61.6)	0	0.21
Hypertriglyceridemia	64 (53.8)	48 (40.3)	7 (5.9)	0.4
Low HDL-cholesterol	11 (50)	11 (50)	0	0.39
Hypercholesterolemia	39 (52)	34 (45.3)	2 (2.7)	0.19
High LDL-cholesterol	22 (42.3)	30 (57.7)	0	0.003
AST	37.2±18.7	39.2±16.8	53.2±21.4	0.12
ALT	77.3±29.9	82.1±20.8	57.4±13.9	0.76
Alkaline phosphatase	216.4±99.1	280±113.9	172.4±115.1	0.3

Data are mean±SD and number (percent). Hypertriglyceridemia defined as a level above the 95th percentile for age and sex, low HDL-cholesterol means a level below the 5th percentile for age and sex, hypercholesterolemia means a level ≥200 mg/dL and high LDL-cholesterol means a level ≥130 mg/dL. *P* values are resulted from one way ANOVA and Chi-square test. HDL: High-density lipoprotein, LDL: Low-density lipoprotein, ALT: Alanine aminotransferase, AST: Serum aspartate aminotransferase, SD: Standard deviation, ANOVA: Analysis of variance, NAFLD: Non-alcoholic fatty liver disease

DISCUSSION

In the natural history of patients with NAFLD is a contrast, that, simple fatty liver patients appear to have benign natural history but patients with steatohepatitis can progress to cirrhosis and liver failure. The presence of fatty liver can found in imaging studies but to identify patients with NASH, liver biopsy is essential.[21] This study was undertaken using enrolled of adults with NAFLD with carefully characterized and uniform entry criteria to determine new insights into the value of routinely obtained clinical and laboratory data for diagnosing the presence and severity of NAFLD. And findings showed that there was no statistically significant relationship between ultrasonograghic findings and steatosis grades, but statistically significant relationship was found between ultrasonograghic findings and fibrosis stage. These findings reveled that, in patients with NAFLD, ultrasonographic finding were not in associate to steatosis but were in relation with fibrosis stage.

Assessment of factors in relation with severity of NAFLD showed that, gender, diabetes, BMI and high LDL-cholesterol are significantly associated with severity of NAFLD. Whereas, patients with definite severe NAFLD were significantly more likely to be female and have diabetes with higher level of BMI compare to mild and moderate NAFLD. And frequencies of high LDL-cholesterol in mild and moderate NAFLD was significantly more compare to severe NAFLD based on ultrasound findings.

In a large cohort study,[22] factors associated with definite NASH in patients with NAFLD and contemporaneous liver biopsies were compared. Authors reported that, patients with NASH were more likely to be women and have diabetes; they also had significantly higher levels of AST, ALT, gamma-glutamyl transpeptidase, triglycerides and lower levels of HDL cholesterol compared to those without definite NASH. But in the present study only high LDL-cholesterol and hypertriglyceridemia were significantly higher in patients with the severe grades of NASH and for other factors these differences were generally not different. Since serum ALT levels are used to screen patients for unsuspected liver disease, but the value of ALT measurements for detecting patients with NASH has been questioned.[16,23-25] Because there is uncertainty regarding how an elevated ALT should be defines. It is reported that laboratory reference ranges for ALT are quite variable, independent of analyzer characteristics and may be unreliable for identifying ALT elevations.[26] Our results support other study.[22] findings that concluded, using any of these upper limits of normal did not provide sufficient sensitivity and specificity to make ALT measurement a reliable screening test to identify NASH in patients with NAFLD.

Identifying early fibrosis may identify patients at risk for progressing to cirrhosis over time. Our results showed that, there were a number of differences in clinical and laboratory parameters associated with the progressive stages of fibrosis but these differences were generally not significant. High LDL-cholesterol was only significant differences in cirrhosis patients compared to other stages or no fibrosis. In several studies, as predictive of the presence of advanced fibrosis, variables that have

Table 4: Comparison of studied variables between subjects in regard to steatosis grade based on liver biopsy findings

Variables	Steatosis grade			*P* value
	Grade 0	Grade 1	Grade 2	
Age (year)	44.3±11.1	42.8±11.1	36.6±3.9	0.33
Male/female (%)	72.2/27.8	63.5/36.5	60/40	0.59
Body mass index (kg/m^2)	27.9±2.6	28.4±3.5	27.5±1.4	0.74
Type 2 diabetes	6 (27.8)	11 (72.2)	0	0.59
Hypertension	14 (35)	26 (65)	0	0.24
Family history of liver	3 (23.1)	9 (69.2)	1 (7.7)	0.76
Hypertriglyceridemia	45 (37.8)	72 (60.5)	2 (1.7)	0.001
Low HDL-cholesterol	9 (40.2)	13 (59.8)	0	0.35
Hypercholesterolemia	28 (37.3)	42 (56)	5 (6.7)	0.17
High LDL-cholesterol	17 (32.7)	28 (53.8)	7 (13.5)	0.003
AST	34.8±18.5	40.3±18.4	32.8±9.6	0.28
ALT	79.5±21.4	77.1±28.9	88±15.8	0.64
Alkaline phosphatase	241.3±105.9	247.5±110.6	246.4±108.3	0.99

Data are mean±SD and number (percent). Hypertriglyceridemia defined as a level above the 95th percentile for age and sex, low HDL-cholesterol means a level below the 5th percentile for age and sex, hypercholesterolemia means a level ≥200 mg/dL and high LDL-cholesterol means a level ≥130 mg/dL. *P* values are resulted from one way ANOVA and Chi-square test. HDL: High-density lipoprotein, LDL: Low-density lipoprotein, ALT: Alanine aminotransferase, AST: Serum aspartate aminotransferase, SD: Standard deviation, ANOVA: Analysis of variance

Table 5: Comparison of studied variables between subjects in regard to fibrosis grade based on liver biopsy findings

Variables	Fibrosis stage			*P* value
	None	Mild/ moderate	Bridging/ cirrhotic	
Age	42.7±11.3	43.7±10.7	38.7±7.4	0.65
Male/female (%)	65.6/34.4	68/32	50/50	0.77
Body mass index (kg/m^2)	27.7±3.5	28.9±2.7	27.8±1.3	0.15
Type 2 diabetes	8 (44.4)	8 (44.4)	2 (11.2)	0.33
Hypertension	18 (45)	20 (50)	2 (5)	0.68
Family history of liver	6 (46.1)	6 (46.1)	1 (7.8)	0.81
Hypertriglyceridemia	64 (53.8)	52 (43.7)	3 (2.5)	0.18
Low HDL-cholesterol	11 (50)	11 (50)	0	0.52
Hypercholesterolemia	39 (52)	31 (41.3)	5 (6.7)	0.42
High LDL-cholesterol	19 (36.5)	28 (53.8)	5 (9.7)	0.011
AST	35.7±18.8	41.4±18.8	32.7±13	0.24
ALT	81.5±29.4	74.4±23.1	83.2±9.5	0.36
Alkaline phosphatase	240.9±97.3	240.7±118.1	250.4±89.1	0.49

Data are mean±SD and number (percent). Hypertriglyceridemia defined as a level above the 95th percentile for age and sex, low HDL-cholesterol means a level below the 5th percentile for age and sex, hypercholesterolemia means a level ≥200 mg/dL and high LDL-cholesterol means a level ≥130 mg/dL. *P* values are resulted from one way ANOVA and Chi-square test. HDL: High-density lipoprotein, LDL: Low-density lipoprotein, ALT: Alanine aminotransferase, AST: Serum aspartate aminotransferase, SD: Standard deviation, ANOVA: Analysis of variance

consistently emerged included the more advanced age and higher prevalence of diabetes with advanced fibrosis, the ratio of AST/ALT, which increases as fibrosis progresses and the relative thrombocytopenia recognized to occur with cirrhosis.[15,27-29] In contrast to these studies present study did not find any significant association between these variables and stage of fibrosis. The differences in methods, sample size and geographical maybe are the causes of different results in these studies.

Perhaps the main limitation of our study is that, because of liver biopsy procedure, being an invasive procedure, which, it is not part of the standard of care to confirm the diagnosis of NAFLD and number of our studied population were not agree to underwent liver biopsy, however, they were similar to other participates for age and sex but we were not able to determine the association between ultrasonograghic findings with liver biopsy findings and other evaluated factors in these excluded patients.

The results of the present study revealed that, in patients with NAFLD, ultrasonographic finding were not in associate to steatosis, but were in relation with fibrosis stage. However, liver biopsy remains the gold standard for establishing steatohepatitis and advanced fibrosis in patients with NAFLD. Other findings from our study were showed that, despite of the significant association between gender (female), diabetes, BMI and high LDL-cholesterol with severity of NAFLD, differences in clinical and laboratory parameters associated with the progressive stages of NAFLD, NASH and fibrosis were generally not significantly different. However, more large studies are needed to confirm and validate these findings.

ACKNOWLEDGMENT

Financial support was provided by the Isfahan University of Medical Sciences (Grant 391006); Isfahan, Iran.

REFERENCES

1. Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. J Hepatol 2010;53:372-84.
2. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, *et al*. Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. Hepatology 2004;40:1387-95.
3. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol 2003;98:960-7.
4. Liangpunsakul S, Chalasani N. Unexplained elevations in alanine aminotransferase in individuals with the metabolic syndrome: Results from the third National Health and Nutrition Survey (NHANES III). Am J Med Sci 2005;329:111-6.
5. Williams R. Global challenges in liver disease. Hepatology 2006;44:521-6.
6. Angulo P. GI epidemiology: Nonalcoholic fatty liver disease. Aliment Pharmacol Ther 2007;25:883-9.
7. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, *et al*. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-21.
8. Brunt EM. Nonalcoholic steatohepatitis. Semin Liver Dis 2004;24:3-20.
9. Szollár L. Fatty liver and global cardiometabolic risk. Orv Hetil 2010;151:1946-50.

10. Byrne CD, Olufadi R, Bruce KD, Cagampang FR, Ahmed MH. Metabolic disturbances in non-alcoholic fatty liver disease. Clin Sci (Lond) 2009;116:539-64.
11. Assy N, Djibre A, Farah R, Grosovski M, Marmor A. Presence of coronary plaques in patients with nonalcoholic fatty liver disease. Radiology 2010;254:393-400.
12. Paschos P, Paletas K. Non alcoholic fatty liver disease and metabolic syndrome. Hippokratia 2009;13:9-19.
13. Tung T, Chiu W, Lin T, Shih H, Hsu C. An exploration of prevalence and associated factors of nonalcoholic fatty liver disease in the taiwanese police service. Iran J Public Health 2011;40:54-62.
14. Obika M, Noguchi H. Diagnosis and evaluation of nonalcoholic fatty liver disease. Exp Diabetes Res 2012;2012:145754.
15. Wieckowska A, Feldstein AE. Diagnosis of nonalcoholic fatty liver disease: Invasive versus noninvasive. Semin Liver Dis 2008;28:386-95.
16. Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, *et al*. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology 2003;37:1286-92.
17. Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni 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;48:792-8.
18. Dowman JK, Tomlinson JW, Newsome PN. Systematic review: The diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2011;33:525-40.
19. Osawa H, Mori Y. Sonographic diagnosis of fatty liver using a histogram technique that compares liver and renal cortical echo amplitudes. J Clin Ultrasound 1996;24:25-9.
20. Strauss S, Gavish E, Gottlieb P, Katsnelson L. Interobserver and intraobserver variability in the sonographic assessment of fatty liver. AJR Am J Roentgenol 2007;189:W320-3.
21. Vuppalanchi R, Unalp A, Van Natta ML, Cummings OW, Sandrasegaran KE, Hameed T, *et al*. Effects of liver biopsy sample length and number of readings on sampling variability in nonalcoholic Fatty liver disease. Clin Gastroenterol Hepatol 2009;7:481-6.
22. Neuschwander-Tetri BA, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, *et al*. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. Hepatology 2010;52:913-24.
23. Kim HC, Nam CM, Jee SH, Han KH, Oh DK, Suh I. Normal serum aminotransferase concentration and risk of mortality from liver diseases: Prospective cohort study. BMJ 2004;328:983.
24. Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: The dionysos nutrition and liver study. Hepatology 2005;42:44-52.
25. Chang Y, Ryu S, Sung E, Jang Y. Higher concentrations of alanine aminotransferase within the reference interval predict nonalcoholic fatty liver disease. Clin Chem 2007;53:686-92.
26. Neuschwander-Tetri BA, Unalp A, Creer MH, Nonalcoholic Steatohepatitis Clinical Research Network. Influence of local reference populations on upper limits of normal for serum alanine aminotransferase levels. Arch Intern Med 2008;168:663-6.
27. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, *et al*. The NAFLD fibrosis score: A noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846-54.
28. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut 2008;57:1441-7.
29. Wieckowska A, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: Present and future. Hepatology 2007;46:582-9.

Source of Support: Nil, **Conflict of Interest:** None declared.