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BRIEF ARTICLE

Noninvasive predictors for liver fibrosis in patients with nonalcoholic steatohepatitis

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Abstract

AIM: To evaluate certain anthropometric, clinical and laboratory features indicating liver fibrosis in nonalcoholic steatohepatitis and to establish the noninvasive markers for liver fibrosis.

METHODS: Eighty-one patients (40 male, 41 female) who were diagnosed with fatty liver by ultrasonographic examination and fulfilled the inclusion criteria participated in the study. Anamnesis, anthropometric, clinical and laboratory features of all cases were recorded and then liver biopsy was performed after obtaining patient consent. Steatosis, necroinflammation and liver fibrosis were examined according to age ⩾ 45, gender, body mass index, central obesity, aspartate aminotransferase (AST)/alanine aminotransferase (ALT) > 1, γ-glutamyltransferase (GGT)/ALT > 1, platelet count, insulin, c-peptide levels and the presence of hypertension, diabetes, hypertriglyceridemia and insulin resistance.

RESULTS: Eighty-one patients with non-alcoholic steatohepatitis (NASH) enrolled in the study. 69 of 81 patients were diagnosed with NASH, 11 were diagnosed with simple fatty liver and 1 was diagnosed with cirrhosis. AST/ALT > 1, GGT/ALT > 11, high serum ferritin and fasting insulin levels, the presence of diabetes, hypertension, hypertriglyceridemia and insulin resistance seemed to enhance the severity of steatosis, necroinflammation and fibrosis but these results were not statistically significant.

CONCLUSION: Liver steatosis and fibrosis can occur in individuals with normal weight. There was no significant concordance between severity of liver histology and the presence of predictors for liver fibrosis including metabolic risk factors.

Key words: Liver fibrosis; Predictors; Nonalcoholic fatty liver disease; Steatohepatitis

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a hepatic pathology which includes fat accumulation and inflammation in hepatocytes accompanied by fibrosis in various degrees, with neglible or no alcohol consumption.

This entity may progress to cirrhosis and liver failure[1]. Although NAFLD is supposed to be in association with certain metabolic disorders like obesity, diabetes and hyperlipidemia, it can also occur in lean individuals and those without diabetes[2,3]. The natural progression of fatty liver is not definitely estimated previously. While approximately 7%-37.6% of patients with non-alcoholic steatohepatitis (NASH) may have advanced fibrosis and 20% of patients with NASH may silently progress to cirrhosis, fatty liver should not always be considered as an innocent condition[4-7]. Due to the difficulties in extensive application of liver biopsy, many anthropometric, clinical and laboratory features in NASH patients were investigated for their currency and worthiness in the prediction of liver fibrosis. Our aim is to research and reveal the validity, accuracy and convenience of certain anthropometric, clinical and laboratory features to predict liver fibrosis and their concordance with liver histology.

MATERIALS AND METHODS

Patients

Eighty-one patients, diagnosed with fatty liver as mild, moderate and severe by ultrasonographic examination and with an elevation in alanine aminotransferase (ALT) levels of at least 1.5 fold of the normal range and persistent liver steatosis, were enrolled in the study in Uludag University Gastroenterology Division. After complete anthropometric, clinical and laboratory assessments, liver biopsy was performed. Exclusion criteria were alcohol consumption ⩾ 20 g/d, pregnancy, positive tests indicating the presence of hepatitis B and C virus, autoimmune liver diseases, hemochromatosis, Wilson's disease, α-1 antitrypsin deficiency, primary biliary cirrhosis, primary sclerosing cholangitis and toxic liver diseases. Data handling and liver biopsy were performed with patient consent. The study was approved by the hospital ethics committee.

Laboratory studies

All cases underwent liver examination by ultrasonography and subsequently anamnesis; anthropometric, clinical, complete blood count and biochemical assessments were performed. Biochemical evaluation consisted of ALT, aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), bilirubin, albumin, high-density lipoprotein (HDL)-cholesterol, triglycerides, fasting glucose and insulin levels and oral glucose tolerance test. Anthropometric measurements included height, weight, body mass index (BMI), waist and hip circumferences and waist/hip ratio values. Diagnosis of obesity was dependent on World Health Organization (WHO) criteria[8]. American Diabetes Association criteria were used to signify type 2 diabetes, impaired glucose intolerance and impaired fasting glycemia. Patients on oral antidiabetics or insulin therapy were accepted as diabetics. Hypertension was recognized when resting blood pressure was ⩾ 140/90 mmHg or when patients were on antihypertensive drug therapy. Triglycerides ⩾ 1500 mg/L were accepted as hypertriglyceridemia. The measurement of insulin resistance was made using homeostatic model assessment (HOMA) method and patients were classed as insulin resistant while HOMA value was ⩾ 2.70. ALT levels 1.5 or more times the upper normal values marked an elevation. The diagnosis of metabolic syndrome was made using WHO criteria[9]: BMI ⩾ 30 kg/m^2, waist/hip circumference ratio > 0.90 in men and > 0.85 in women, fasting blood glucose ⩾ 1100 mg/L, overt diabetes, presence of impaired glucose tolerance and/or IR, triglycerides ⩾ 1500 mg/L, HDL-cholesterol < 400 mg/L in men and < 500 mg/L in women, arterial blood pressure ⩾ 140/90 mmHg and presence of microalbuminuria. Patients had at least three of these criteria to be diagnosed with metabolic syndrome. The study was approved by the hospital ethics committee.

Pathology

All 81 patients underwent liver biopsy according to the severity of clinical and laboratory features and patient consent. Liver biopsy specimens were examined by liver pathologists in the Department of Pathology at the Medical Faculty in Uludag University. Necroinflammation and fibrosis in liver were evaluated using the histopathological criteria defined by Brunt *et al*[10]. Diagnosis of NASH was dependent on steatosis (mild: < 33% of lobules, moderate: 33%-66% of lobules and severe: > 66% of lobules) and 2 of the 3 features: (1) necroinflammation with mononuclear cells and/or polymorphonuclear leucocytes; (2) ballooning degeneration of hepatocytes, Mallory bodies; and (3) pericellular, perisinusoidal and/or bridging fibrosis. Steatosis and necroinflammation were categorized as grade 1, 2 and 3 and fibrosis as grade 1, 2, 3 and 4 (cirrhosis).

Statistical analysis

Statistical significance was not reached due to the small number of patients and statistical evaluation and *P* values were not available. Evaluations were performed using percentage values. Hence, patient features were evaluated according to their percentage values.

RESULTS

Anthropometric, clinical and laboratory results

Eighty-one patients (40 male, 41 female) who were diagnosed with fatty liver by ultrasonographic examination participated in the study at Uludag University Gastroenterology Division. All patients underwent liver biopsy. 69 (35 male, 34 female) of 81 patients were diagnosed with NASH, 11 (4 male, 7 female) were diagnosed with simple fatty liver and 1 (male) was diagnosed with cirrhosis. Initial characteristics of all patients were obtained and recorded as shown in Table 1. We used the most pronounced independent risk factors such as age ⩾ 45 years, gender, BMI > 30 kg/m^2, central obesity (waist/hip ratio

Table 1 General aspects of all non-alcoholic steatohepatitis cases *n* (%)

Nonalcoholic steatohepatitis (*n* = 70)	
Average age (yr)	47.9 ± 8.74
Gender (male/female)	36/34
Hepatomegaly	16 (23.9)
BMI (kg/m^2)	30.4 ± 4.79
Normal weight (BMI < 24.9 kg/m^2)	3 (4.30)
Overweight (BMI = 25-29.9 kg/m^2)	34 (48.5)
Obese (BMI = 30-39.9 kg/m^2)	29 (41.4)
Morbid obese (BMI > 40 kg/m^2)	4 (5.80)
Waist/hip cir (E > 0.90, K > 0.85)	49 (71)
Systolic blood pressure (mmHg)	124 ± 16.2
Diastolic blood pressure (mmHg)	75.6 ± 11.9
Hypertension	21 (30.4)
HDL-cholesterol (mg/dL)	46.3 ± 8.07
Low-HDL-cholesterol	28 (40.5)
Triglycerides (mg/dL)	163 ± 79.5
Hypertriglyceridemia	36 (52.1)
Fasting glucose (mg/dL)	107 ± 26.2
Diabetes mellitus	20 (28.8)
Fasting insulin (μU/mL)	16.6 ± 13.0
Homeostatic model assessment-insulin resistance value	3.91 ± 2.45
Fasting c-peptid	4.35 ± 2.15
aspartate aminotransferase (U/L)	48.2 ± 23.9
Alanine aminotransferase (U/L)	76.2 ± 35.2
Gama glutamyl transpeptidase (U/L)	59.2 ± 46.8
Alkaline phosphatase (U/L)	93.4 ± 32.3
İnsulin resistance	30 (43.4)
Metabolic syndrome	46 (66.6)

This table shows that numbers of non obese patients are much higher than obese patients and 33.4% of non-alcoholic steatohepatitis patients have no metabolic syndrome. BMI: Body mass index; HDL: High-density lipoprotein.

> 0.90 in men and > 0.85 in women), AST/ALT > 1, GGT/ALT > 1, platelet count, fasting serum levels of ferritin, c-peptid and insulin and presence of hypertension, diabetes, hypertriglyceridemia and insulin resistance.

Table 1 shows that the numbers of normal and overweight patients were higher than those of obese and morbidly obese patients. This indicates that the risk for liver steatosis could be higher when BMI values exceed 25 kg/m^2. Prevalences of other components of metabolic syndrome were not significantly increased, as stated in Table 1. These results indicate that NASH could occur in patients with only one risk factor or even in patients without any risk factors.

Although the simple fatty liver group is small (11 patients), we determined that two patients had 2, seven patients had 3, one patient had 4 and one patient had 5 risk factors. These results have shown that the presence and numbers of metabolic risk factors did not give information about liver histology. It seems that discrimination between NASH and simply fatty liver will not be made according only to clinical, epidemiological, anthropometrical or laboratory results.

Furthermore, the presence of predictors for liver fibrosis were also searched for in patients with simple fatty liver. However, in patients with age ⩾ 45, obesity, hypertension, diabetes and hypertriglyceridemia, it seemed to be increased but these results were not significant.

Table 2 shows that female gender, age > 45 years seem to have severe steatosis and necroinflammation. Interestingly, patients with normal BMI seem to have severe steatosis and necroinflammation. However, AST/ALT > 1, GGT/ALT > 1 and low platelet count, increase in fasting serum ferritin, insulin and c-peptide levels and presence of metabolic risk factors (diabetes, hypertriglyceridemia, hypertension and insulin resistance) seem to increase severe steatosis and necroinflammation but due to the small number of cases, these results are not significant.

Table 3 shows that gender and age > 45 did not seem to influence the development of fibrosis significantly. Patients with normal BMI seemed to have severe fibrosis but because of the small number of cases, these results were not significant. Central obesity, AST/ALT > 1, GGT/ALT > 1, elevated serum ferritin and fasting insulin levels, presence of hypertension, diabetes, hypertriglyceridemia and insulin resistance seemed to increase fibrosis but these findings were not significant. We diagnosed one patient with cirrhosis who had normal AST/ALT and AST/GGT ratio, normal platelet count and serum ferritin, fasting insulin and c-peptide levels. This patient had no metabolic risk factors apart from diabetes.

Histopathology

The detailed features of liver histology of our 81 cases were examined using the presence of "predictors for liver fibrosis". Tables 2 and 3 present the influences of predictors on liver histology in our 70 NASH cases. The remaining 11 patients were diagnosed with simple fatty liver.

DISCUSSION

In the present study, we aimed to reveal simple, confident and feasible noninvasive parameters to assign the severity of nonalcoholic steatohepatitis. General aspects of our patients are presented in Table 1.

Advanced obesity was stated as a risk factor for the development of liver fibrosis by Sobhonslidsuk *et al*[11], Ong *et al*[12] and Ratziu *et al*[13]. Recent studies revealed that the people with normal body weight but high visceral fat ratio (central obesity) could have NAFLD, metabolic syndrome and insulin resistance[2,3]. Sobhonslidsuk *et al*[11] and Cheung *et al*[14] stated that abdominal obesity correlated only with liver inflammation and so waist circumference predicts metabolic risk condition with the most significance. Angelico *et al*[15] and Marchesini *et al*[16] found a correlation between various degrees of liver steatosis and BMI but we did not detect any relationship between steatosis and BMI (Table 2). Boza *et al*[17] found no significant association between BMI and histological changes. In the latest study, high HOMA-IR values and ALT levels were the only independent predictors of NASH. Gholam *et al*[18] stated that except for BMI and hyperglycemia, insulin resistance and the metabolic syndrome were associated with the presence of NASH and fibrosis. Rocha

Table 2 Predictors of liver fibrosis and liver histology (steatosis and necroinflammation) in patients with nonalcoholic steatohepatitis

	Steatosis (n = 70)			Necroinflammation (Grade) (n = 70)		
	Mild (%)	Moderate (%)	Severe (%)	Mild (%)	Moderate (%)	Severe (%)
Gender						
Male	38.9	38.9	22.1	36.1	55.5	8.3
Female	41.2	35.3	23.5	20.6	64.7	14.7
Age (yr)						
> 45	35.4	37.5	27.0	25.0	62.5	12.5
< 45	45.4	36.3	18.1	36.3	59.0	4.5
Body mass index (kg/m^2)						
18.5-24.9	0	66.6	34.4	33.3	33.4	33.3
25.0-29.9	34.2	42.9	22.9	31.4	60.0	8.6
30.0-39.9	50.0	21.5	28.5	32.1	53.6	14.3
> 40	25.0	75.0	0	50.0	50.0	0
Central obesity						
+	40.0	38.0	22.0	28.5	61.2	10.2
-	35.0	35.0	30.0	28.5	57.1	14.2
AST/ALT > 1	40.0	20.0	40.0	20.0	40.0	40.0
AST/ALT < 1	37.5	39.0	23.4	29.6	60.9	9.3
GGT/ALT > 1	36.3	27.2	36.3	31.8	50.0	18.1
GGT/ALT < 1	38.2	42.5	19.1	25.5	65.9	8.6
Platelet count						
Low	33.4	0	66.6	33.4	33.3	33.4
Normal	39.3	37.8	22.7	36.3	53.0	10.6
Ferritin						
Elevated	0	33.4	66.6	0	33.4	66.6
Normal	39.3	37.8	22.7	28.7	62.1	10.6
Fasting insulin						
Elevated	14.4	28.5	57.1	14.4	71.4	14.4
Normal	42.0	35.5	22.5	30.6	62.0	11.2
Fasting c-peptid						
Elevated	31.2	37.5	31.2	15.6	71.8	12.5
Normal	45.9	35.1	18.9	37.8	51.3	10.8
Hypertension						
Present	38.1	33.4	28.5	19.0	62.0	19.0
Absent	38.7	38.7	22.4	32.6	59.2	8.2
Diabetes						
Present	38.1	33.4	28.5	23.8	57.1	14.2
Absent	36.7	38.7	24.4	28.5	61.2	10.2
Hypertriglyceridemia						
Present	36.2	38.8	25.0	19.4	66.6	13.8
Absent	41.0	35.2	23.5	38.2	52.9	8.8
Insulin resistance						
Present	37.9	34.4	27.5	24.1	65.5	10.3
Absent	40.0	40.0	20.0	50.0	40.0	10.0

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyltransferase.

et al[19] said that the prevalence of NAFLD was 2.3% in 199 patients with central obesity. Nevertheless, we observed no connection between central obesity and fibrosis/necroinflammation. It is a remarkable point that necroinflammation and fibrosis could progress even in patients with normal weight (Tables 2 and 3).

Females aged over 45 years were considered to influence liver histology in nonalcoholic steatohepatitis[20]. Daryani *et al*[21] and Shimada *et al*[22] have found that females over 55 had a relationship with liver steatosis and advanced fibrosis and other metabolic parameters (e.g. obesity, diabetes, hypertension) but these were not statistically significant predictors[23]. Harrison *et al*[24] and Yatsuji *et al*[25] said that advanced age was related to advanced fibrosis. Singh *et al*[26] and Liew *et al*[27] found that older females were identified as independent predictors of fibrotic severity. Ong *et al*[12], Helling *et al*[28] and Arun *et al*[29] stated that male gender was associated with NASH. Prashanth *et al*[30] said that older age, duration of diabetes mellitus, degree of glycemic control, BMI, waist circumference and family history of diabetes mellitus did not predict the presence or severity of NAFLD or fibrosis. In our study, age over 45 years and gender did not seem to have more severe steatosis/necroinflammation and fibrosis (Tables 2 and 3).

Elevated ALT and AST levels were assessed as remarkable markers for NASH and liver fibrosis. Hossain *et al*[31] found that diabetes mellitus and aminotransferase levels are independent predictors of fibrosis in NAFLD. Chavarría-Arciniega *et al*[32] stated that fibrosis showed correlation only with AST. Rodríguez-Hernández *et al*[33] stressed that ALT was correlated with inflammation and

Table 3 Predictors of liver fibrosis and histological stage in nonalcoholic steatohepatitis cases (n = 70)

	Absent	Fibrosis (%)		Bridging	Cirrhosis (%)
		Perisinusoidal/ pericellular	Periportal		
Gender					
Male	50.0	22.2	16.6	8.3	2.7
Female	38.2	47.0	5.9	8.9	-
Age (yr)					
> 45	45.8	39.7	6.25	6.25	2.1
< 45	41.0	22.7	22.7	13.6	-
Body mass index (kg/m^2)					-
18.5-24.9	33.3	0	33.3	33.3	
25.0-29.9	44.4	41.6	8.3	5.5	-
30.0-39.9	50.0	21.4	14.2	10.7	3.5
> 40	25.0	75.0	0	0	-
Central obesity					
Present	38.0	36.0	16.0	8.0	2.0
Absent	60.0	30.0	0	10.0	-
AST/ALT > 1	20.0	20.0	20.0	40.0	-
AST/ALT < 1	53.1	29.6	10.9	4.6	1.5
GGT/ALT > 1	52.1	26.0	13.0	8.63	-
GGT/ALT < 1	54.3	30.4	8.6	6.5	2.1
Platelet count					
Low	66.6	33.4	0	0	0
Normal	44.0	35.0	10.5	9.0	1.5
Ferritin					
Elevated	33.4	0	66.6	0	0
Normal	44.0	36.5	9.0	9.0	1.5
Fasting insulin					
Elevated	28.5	14.5	28.5	28.5	-
Normal	43.6	37.1	9.7	8.0	1.6
Fasting c-peptid					
Elevated	59.0	27.2	6.8	6.8	-
Normal	44.0	32.0	16.0	8.0	2.3
Hypertension					
Present	28.5	52.3	9.5	9.5	-
Absent	53.0	26.5	12.2	6.1	2.0
Diabetes					
Present	19.0	47.6	14.2	14.2	4.7
Absent	55.1	28.5	10.2	6.1	-
Hypertriglyceridemia					
Present	38.8	33.4	16.6	8.3	-
Absent	47.0	38.2	5.8	5.8	3.0
Insulin resistance					
Present	37.0	40.7	18.5	3.7	3.7
Absent	77.2	13.6	-	9.2	-

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyltransferase.

fibrosis. Prashanth *et al*[30] also said that serum alanine aminotranferase (ALT) and ALP levels were significantly higher in patients with steatohepatitis. Gholam *et al*[18] also said that elevated transaminase levels correlated with NASH and fibrosis. However, 46% of their subjects with NASH had normal transaminases. Shi *et al*[34] denoted that elevated serum level of ALT is an independent predictor of the degree of inflammation but not of steatosis and fibrosis. Nevertheless, recent certain studies expressed that ALT and AST were not reliable markers for NASH or for fibrosis[34,35]. Ong *et al*[12] said that waist/hip ratio and AST were independently associated with advanced fibrosis but in the latest study the majority of the patients with either NASH or advanced fibrosis had normal AST. Nomura *et al*[36] and Mofrad *et al*[37] stated that significant liver disease could exist with normal liver enzyme levels. Fracanzani *et al*[38] also stressed that normal ALT is not a valuable parameter to exclude patients from liver biopsy. We also found that there was no relationship between ALT and AST levels and liver fibrosis.

Furthermore, the ratio of AST/ALT higher than 1 was asserted to be a marker for liver fibrosis in NASH. Prashanth *et al*[30] found that serum AST/ALT ratio was significantly higher in patients with severe fibrosis and, additionally, all patients with severe fibrosis had metabolic syndrome. Gramlich *et al*[20], Harrison *et al*[24] and McPherson *et al*[39] said that the presence of AST/ALT > 1 could exclude liver fibrosis in patients with NAFLD. Myers[35] and Amarapurkar *et al*[40] revealed that elevation in AST,

ALT levels and AST:ALT > 1 did not show significant association with fibrosis. In the latest study, there was no accurate noninvasive method available that could previously determine the risk of fibrosis in patients with NASH and the elevated levels of transaminases were non-specific with the disease. Hence, liver biopsy remains the gold standard in staging and predicting progression in patients with NASH. Myers[35] said that an AST/ALT ratio above 1 might indicate advanced fibrosis; however, its sensitivity was poor. Bahrami *et al*[41] determined that the value of AST/ALT < 1 was present in 65.3% of patients with NAFLD. Shimada *et al*[22] said that the AST: ALT ratio was less specific. In our study, the presence of AST/ALT > 1 seemed to increase steatosis, necroinflammation and fibrosis, but these results were not significant. Furthermore, 20% of patients with AST/ALT > 1 had no fibrosis, the other 20% of patients with AST/ALT > 1 had only slight necroinflammation. In addition, in patients with AST/ALT < 1 the rate of fibrosis was approximately 47%. For example, in our study, one patient with cirrhosis was male, 55 years old and had AST/ALT < 1 but no metabolic syndrome. We observed that the presence of GGT/ALT > 1 also had no relationship with liver histopathology.

Metabolic disorders are considered to influence liver histology in NAFLD[42,43]. Prashanth *et al*[30] pointed out that the prevalence of NASH increased with the components of metabolic syndrome. Diabetes mellitus influences liver histology in NAFLD[40,44]. Singh *et al*[26] signified that female gender, BMI, waist:hip ratio, hypercholesterolemia and LDL levels are independent predictors of liver damage in patients with NASH. According to Helling *et al*[28], only increased triglycerides and decreased prealbumin correlated with NASH. Assy *et al*[45] defined hypertriglyceridemia and diabetes as the only risk factors that increase the risk of fatty infiltration in hyperlipidemic patients. Liew *et al*[27] featured that serum cholesterol and low-density lipoprotein cholesterol levels were risk factors associated with gallbladder disease and fatty liver disease. Rodríguez-Hernández *et al*[33] said that diabetes and ALT correlated with histological hepatic changes. Amarapurka *et al*[44] found that diabetes mellitus does not always precede NASH and risk factors like central obesity, dyslipidemia and family history do not forebode the occurrence of NASH in diabetic patients. We also did not detect any significant correlation between individual metabolic risk factors and liver fibrosis in NASH patients (Tables 2 and 3). Moreover, in our study one patient, female and over 45 years, with NASH had no metabolic risk factor.

Ryan *et al*[42] said that except for defined metabolic syndrome, other individual features of metabolic syndrome did not correlate with hepatic fibrosis. Nonetheless, all patients with NASH did not fulfil the criteria of metabolic syndrome and liver histology, even in association with metabolic syndrome which has always represented severe steatohepatitis or advanced fibrosis. Kim *et al*[3] stated that NAFLD could occur in non obese and non diabetic individuals. The prevalences of metabolic syndrome were 40% in men and 26% in women according to Hamaguchi *et al*[46]. Pagano *et al*[47] defined the prevalence of metabolic syndrome in NASH cases as 47%. Lizardi-Cervera *et al*[48] signified the prevalence of metabolic syndrome in patients with NAFLD as 22.8%. Moon *et al*[49] studied whether metabolic risk factors had a relationship with the stage of liver fibrosis but no significant differences between histological features in NASH patients with or without metabolic syndrome were found. Kang *et al*[43] stated that as low a proportion of 34% of NAFLD patients had metabolic syndrome. Xanthakos *et al*[50] stressed that in morbidly obese adolescents, severe NASH was uncommon and the presence of metabolic syndrome did not distinguish NASH from steatosis alone. In our study, severity of steatosis, necroinflammation and fibrosis were not significant different in NASH patients with metabolic syndrome when compared to those without it.

Fasting c- peptid and insulin levels were asserted to tend to increase in NAFLD. Patients with fatty liver seem to have lower c-peptid and insulin levels than those with NASH[47,51]. Recent studies claimed that metabolic risk factors and insulin resistance could influence liver histology[52]. Sobhonslidsuk *et al*[11] represented that insulin resistance and elevated visceral fat are risk factors for the presence of NASH. Gholam *et al*[18] said that individuals with NASH had a high level of insulin resistance when compared to those with simple fatty liver. The prevalence of insulin resistance was 85% in the study by Willner *et al*[53]. But Marchesini *et al*[54] revealed the prevalence of insulin resistance in NAFLD was 61%.

However, Dixon *et al*[55] reported that HOMA-IR value, ALT and arterial hypertension were independent predictors for NASH, but they also found that 7.8% of their study patients had NASH even although they had normal AST and HOMA-IR values. Bahrami *et al*[41] found the rate of insulin resistance was only 54.7% in 53 patients with NASH. Guidorizzi de Siqueira *et al*[52] said that insulin resistance was detected in only 33% of NAFLD patients but there was a high frequency of IR in patients with advanced fibrosis. Sakurai *et al*[56] have specified that only steatosis was significantly and independently associated with elevated HOMA values but there was no similar association with the grade or stage of NASH.

An interesting observation expressed by Machado *et al*[57] is that the rates of insulin resistance in NAFLD patients could vary from 47% to 98% and in their study, 36% fulfilled three criteria of metabolic syndrome. We detected that, although high c-peptid and insulin levels and presence of insulin resistance seemed to increase the severity of steatosis, steatohepatitis and liver fibrosis, the findings were not significant.

Low platelet count was proposed to be a marker of fibrosis according to Shimada *et al*[22] and Stepanova *et al*[58]. According to the literature, this parameter was not significant alone as a combination of fibrosis markers are advisable[19,59-62]. In the present study, the relationship between

low platelet count and the severity of steatosis, steatohepatitis and fibrosis were not significant.

Licata *et al*[63] pointed out that high serum ferritin level is a risk factor for steatosis. Fracanzani *et al*[38] said that fibrosis was independently associated with elevated serum ferritin and normal ALT level is not a reliable parameter to exclude patients from liver biopsy. However, Loguercio *et al*[64] revealed that abnormal GGT or ALT, age and ferritin were associated with steatosis but that no single factor was found to be an independent predictor. Pagano *et al*[47] also said that parameters related to iron metabolism did not differ when comparing patients with NASH to the control group. Dixon *et al*[55] represented that there was no difference in ferritin levels between patients with NASH and without NASH. Friis-Liby *et al*[65] stated that abnormalities in iron indices were detected in 31 patients of 102 (39%) and elevated ferritin in 29 patients of 102 (28.4%). Pagadala *et al*[59] signified that elevated serum ferritin in NAFLD has not been confirmed by other studies. Chitturi *et al*[51], Angulo *et al*[62] and Younossi *et al*[66] did not observe any relationship between iron metabolism and the clinical or pathological outcomes in patients with NAFLD. Nevertheless, in our study high ferritin levels seemed to raise steatosis, necroinflammation and fibrosis but these results were not significant.

In conclusion, none of the present tools yield all that is needed for the "perfect" fibrosis marker as each non invasive predictor lacks accuracy and reliability and hence, combination algorithms of fibrosis markers are needed[35,59,67,68]. Although noninvasive, simple, reproducible and reliable biomarkers are still greatly needed, none of them can substitute for a liver biopsy[62,69,70].

COMMENTS

Background

Nonalcoholic fatty liver disease is a condition which is described as fat accumulation, especially triglycerides, in liver cells. This condition may lead the function of liver to deteriorate. So liver diseases including liver cirrhosis and liver failure may occur. Hence to detect this fat accumulation and to predict the probable evil outcomes are very important.

Research frontiers

To avoid the harmful consequences of NAFLD apart from liver biopsy many noninvasive methods were improved. These methods are generally based on blood tests. The studies on noninvasive methods are still going on to determine the most sensitive and specific tools. The authors also searched simple, available, applicable and reliable methods.

Innovations and breakthroughs

New predictive markers should be investigated before severe outcomes of NAFLD have occured. This study aimed to reveal and establish simple, available and accurate noninvasive markers for liver fibrosis which are applicable in every health center.

Applications

If predictive tools could be applicable even in small health centers with high accuracy well then they are really reliable so people with fatty liver can have a check-up for prognosis of liver steatosis. The variables we used to predict liver fibrosis are even though usable and beneficial in health centers but still more investigations are needed.

Terminology

Fatty liver or liver steatosis can occur without alcohol consumption and progress to steatohepatitis which means an inflammation in liver then this condition can deteriorate so fibrous tissue begin to form after liver cell destruction. Once liver cell destruction begins somehow the event may progress to cirrhosis and liver failure. Hence to prevent this process is now an important problem and early determination of coming hazardous results of NAFLD became remarkable and developing issue in whole liver diseases.

Peer review

This descriptive study suggest that follow-up of the individuals with fatty liver should not be neglected. Especially studies on simple, accurate, reliable and above all applicable noninvasive markers for liver fibrosis in every health center should go on.

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