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The prevalence of celiac disease among patients with non-alcoholic fatty liver disease in Iran

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Background/aims: *Some patients with non-alcoholic fatty liver disease have no obesity-related etiologies. Celiac disease could potentially present with elevated liver enzymes and chronic liver disease. The aim of this study was to evaluate the prevalence of celiac disease among patients with non-alcoholic fatty liver disease.* ***Methods:*** *Three hundred sixteen patients defined as non-alcoholic fatty liver disease based on elevated transaminases, liver ultrasound and/or liver biopsy were enrolled. Body mass index, waist circumference and symptoms were recorded. All were tested for recombinant IgA anti-tissue transglutaminase antibody and total IgA level. In patients with positive serology for anti-tissue transglutaminase, IgA class endomysial antibody values were determined with a commercially available indirect immunofluorescence method, and then endoscopy with duodenal biopsies was performed.* ***Results:*** *The mean age of patients was 40.56±11.48 years and 50.9% were female. Celiac disease was confirmed in 7 patients (2.2%). Of these, all had body mass index between 18.37 and 26.91 kg/m^2. Celiac disease was more commonly diagnosed among non-alcoholic fatty liver disease patients with body mass index <27 kg/m^2 compared to patients with body mass index >27 kg/m^2 (5.83% vs. 0%; p=0.001).* ***Conclusions:*** *The prevalence of celiac disease among patients with non-alcoholic fatty liver disease is significantly higher than what was previously reported in the general population of Iran; thus, screening for celiac disease in these patients is reasonable, particularly in patients with body mass index <27 kg/m^2.*

Key words: Non-alcoholic fatty liver disease, celiac disease, body mass index

İran'da alkole bağlı olmayan yağlı karaciğer hastalarında çölyak hastalığı sıklığı

Amaç: *Alkole bağlı olmayan yağlı karaciğer hastalarının bir kısmında etyolojide obezite bulunmaz. Çölyak hastalığı, karaciğer enzimlerinde yükselmeye neden olabilir ve kronik karaciğer hastalığına eşlik edebilir. Bu çalışmanın amacı non-alkolik yağlı karaciğer hastalarında çölyak hastalığı prevalansını araştırmaktır.* ***Yöntem:*** *Alkole bağlı olmayan yağlı karaciğer tanısı ultrasonografi, yüksek karaciğer enzimleri ve karaciğer biyopsisi ile konan 316 hasta incelendi. Vücut kitle indeksi, bel çevresi ve semtomlar kaydedildi. Tüm hastalarda anti-doku transglutaminaz Ig A antikoru ve total Ig A seviyeleri ölçüldü. Antikor pozitif olan hastalarda anti-endomysial antikor bakıldı. Endoskopi yapıldı ve duodenal biyopsiler alındı.* ***Bulgular:*** *Hastaların yaş ortalaması 40,56±11,48 ve %50,9'u kadındı. Yedi vakada (%2,2) Çölyak hastalığı tespit edildi. Bu hastaların vücut kitle indeksleri 18,37 kg/m^2 ile 26,91 kg/m^2 arasındaydı. Çölyak hastalığı sıklığı vücut kitle indeksi 27 kg/m^2'nin altında olanlarda olmayanlara göre anlamlı olarak yüksek (%5,83 - %0; p=0,001) tespit edildi.* ***Sonuç:*** *İran'da Alkole bağlı olmayan yağlı karaciğer hastalarında Çölyak hastalığı sıklığı genel populasyona göre anlamlı yüksek bulundu. Bu çalışmanın sonuçlarına göre vücut kütle indeksi 27 kg/m^2'nin altında Alkole bağlı olmayan yağlı karaciğer hastalarında çölyak hastalığının araştırılması akılcıdır.*

Anahtar kelimeler: Alkole bağlı olmayan yağlı karaciğer hastalığı, çölyak hastalığı, vücut kitle indeksi

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common disease of unknown origin characterized by histologic features similar to alcoholic-like liver injury but in the absence of significant alcohol intake. NAFLD includes a wide spectrum of liver pathology – from steatosis alone, through the necroinflammatory disorder of nonalcoholic steatohepatitis (NASH), to cirrhosis and liver cancer. Aside from the pathology and excluding other causes of FLD, a key defining issue is the threshold of alcohol consumption <20–40 g ethanol/day (1,2). Abnormal liver function tests (LFTs) typically comprise minor (1.5- to 5-fold) elevations of alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT). Hepatic ultrasound in NAFLD shows increased echogenicity, or 'bright liver' and blurring of vessels. NAFLD is frequently associated with disorders such as insulin resistance, obesity, type 2 diabetes mellitus, hyperlipidemia, protein-calorie malnutrition, and jejunoileal bypass surgery; however, in a subgroup of NAFLD patients, the true relevant cause remains undetermined. Celiac disease (CD) is a common immune-mediated disorder that affects approximately 1% of Caucasians and develops in genetically susceptible subjects after the ingestion of gluten proteins. From a histopathological standpoint, it is characterized by villous atrophy and crypt hyperplasia of the small intestinal mucosa (3). In previous epidemiologic studies in Iran, a prevalence rate of 1/164 has been reported in blood donors (4).

Celiac disease (CD) has been found in about 10% of patients with unexplained abnormal liver tests, and in about 3.5% of patients with NAFLD as the only manifestation of the disease (5,6). The frequency of subclinical or silent presentations in older children and adults highlights the importance of CD screening in patients with unexplained chronic abnormal LFTs and NAFLD without any specific etiology.

We aimed to determine the spectrum of CD among Iranian patients primarily diagnosed as NAFLD.

MATERIALS AND METHODS

From October 2008 to November 2009, 316 patients primarily diagnosed as NAFLD, based on an echogenic ('bright') liver ultrasonogram (USG) with or without abnormal liver tests of at least 6 months duration and/or liver biopsy were recruited from the outpatient Liver Diseases Clinic of Imam Khomeini Hospital affiliated with Tehran University of Medical Sciences. All patients gave informed consent prior to enrollment. The ethical committee of the university approved the study protocol.

The following criteria were applied to exclude the most frequent etiologies of NAFLD:

a. Viral: HBsAg and HBV-DNA negative; anti-HCV and HCV-RNA negative; human immunodeficiency virus (HIV)-negative (by ELISA antibody)

b. Autoimmune: ANA, AMA, SMA, LKM assay negative (indirect immunofluorescence)

c. Metabolic: normal serum levels of ceruloplasmin and ·1-antitrypsin

d. Toxic: alcohol intake <20 g per day, no current or past chronic drug use, no professional exposure to hepatotoxic substances.

Anthropometric measurements included body mass index (BMI, kg/m^2) and determination of abdominal girth (waist circumference with patient standing, halfway between lower ribcage and iliac crest). Central obesity in Asians is defined as waist circumference ≥90 cm for men and ≥80 cm for women (7), and considering this criterion, patients were divided into two groups as normal waist circumference and increased waist circumference. Metabolic tests including fasting serum lipids (triglyceride, cholesterol) and lipoprotein levels (high-density lipoprotein [HDL] cholesterol, low-density lipoprotein [LDL] cholesterol), fasting blood glucose and hemoglobin, and blood cell counts were recorded.

All patients were tested by ELISA for IgA anti-tissue transglutaminase (anti-tTG) using human recombinant tTG from human embryonic kidney cell line 293-EBNA (Eurospital SpA; Trieste, Italy), and values >7 U/ml were considered positive as recommended by the manufacturer. To exclude IgA deficiency, total serum IgA concentrations were also measured in all patients. No cases yielded IgA levels <0.05 g/L, indicative of selective IgA deficiency, and the values obtained were within the reference range (>1.24 g/L). In patients with positive serology for anti-tTG, IgA class endomysial antibody (EmA) values were determined with a commercially available indirect immunofluorescence method using a standard method. Then, patients with positive results for any of these tests underwent endoscopy and biopsy of the second part of the duodenum for definite diagnosis.

Statistical Analysis

Data were expressed as percentages or absolute numbers for categorical variables and means and standard deviations (SD) for continuous variables. Comparisons were made using the Mann-Whitney U test for quantitative variables and chi-square test and Fisher exact test for categorical variables. A p value of <0.05 was considered to be statistically significant. Statistical analysis was performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The mean age of the study patients was 40.56±11.48 years and 50.9% were female. The mean value of BMI in all subjects was 27.8±3.05 kg/m^2, and the mean waist circumference was 100.05±12.62 cm and 96.90±13.99 cm in males and females, respectively. Of all patients, 15.5% (49/316) had BMI <25 kg/m^2 and 17.7% (56/316) had normal waist circumference based on Asian criteria. Thirty-four patients (10.8%) had overlapping feature of both normal waist circumference and BMI value <25 kg/m^2.

Eight out of 316 patients (2.5%) were IgA anti-tTG-positive, and 7 were positive for IgA anti-EmA. IgA anti-tTG values ranged from 7.8 to 122.7 U/ml among EmA-positive patients, and 1 EmA-negative patient had a value of 7.2.

Seven patients (2.2%) with positive serology for both antibodies had histopathologic features of CD according to the modified Marsh classification, but 1 patient with 1 positive serology for IgA anti-tTG had normal histology. Histologies in 3 patients were compatible with Marsh IIIA, 2 with Marsh II and 2 with Marsh I.

Overall, CD was diagnosed in 7 patients (2.2%; 4 female [57%]). The difference between mean age of CD patients (33.57 ± 8.24 years) and of non-CD patients (40.68 ± 11.38 years) was not statistically significant. CD patients had BMI values between 18.37 and 26.91 kg/m^2. There were statistically significant differences in BMI values between NAFLD patients with CD (22.98±2.93 kg/m^2) and NAFLD patients without CD (27.91±2.97 kg/m^2) (p<0.001). Considering only subjects with BMI value <27 kg/m^2, the prevalence of CD was 5.8% (7/120). In subjects with normal waist circumference, 7.1% (4/56) had CD, whereas in the group with abnormal waist circumference based on Asian criteria, 1.2% (3/260) had CD, and the difference was statistically significant (p=0.021) (Table 1). All CD patients with normal waist circumference had BMI value <25 kg/m^2, but of 3 patients with abnormal waist circumference, 1 had BMI value <25 kg/m^2 (Table 2).

Comparing laboratory features between CD patients and non-CD patients, hemoglobin level and triglyceride level showed statistical differences (p=0.006 and 0.028, respectively), although mean triglyceride level in both groups were >150 mg/dl (Table 3).

Four CD patients were anemic, with respect to normal ranges in males and females. CD patients had elevated liver enzymes, which after a 6-month follow-up with a gluten-free diet (GFD), dropped to <40 IU/L. Three patients underwent post-treatment liver biopsy, in which steatosis disappeared. Two patients with CD had complaints of bloating and intermittent loose stool, which significantly improved with GFD. The other five patients were completely asymptomatic.

DISCUSSION

In the studied NAFLD patients, the mean age of 40.56±11.48 years and slight female predominance (50.9%) are compatible with previous studies that typically recognized NAFLD in the 4th–5th decade of life (8). Earlier studies suggested a female

Table 1. Frequencies of celiac disease among NAFLD patients based on waist circumference (WC) (normal: <90 in males, <80 cm in females)

			NAFLD		
			Without celiac disease	With celiac disease	Total
WC	Normal	Count	52	4	56
		%	92.9%	7.1%	100%
	Abnormal	Count	257	3	260
		%	98.8%	1.2%	100%
[1]P value			0.021		

Table 2. Diagnosed celiac disease among NAFLD patients identified by BMI and waist circumference (normal: <90 cm in males, <80 cm in females)

			Waist Circumference		
			Normal	Abnormal	Total
BMI	18-24.99	Count	4	1	5
		% of Total	57.1%	14.3%	71.4%
	25-29.99	Count	0	2	2
		% of Total	0.0%	28.6%	28.6%
Total		Count	4	3	7
		% of Total	57.1%	42.9%	100.0%

Table 3. Laboratory features of NAFLD patients with and without celiac disease

	NAFLD with CD		NAFLD without CD		P value
	Mean	SD	Mean	SD	
AST (U/L)	71.57	32.94	56.05	22.93	[1]NS
ALT (U/L)	82.14	32.16	70.15	24.72	NS
ALP (U/L)	198.57	92.76	187.8	50.65	NS
FBS (mg/dl)	87.29	11.83	93.98	18.27	NS
TG (mg/dl)	197.7	51.57	265	87.88	0.028
Cholesterol (mg/dl)	215.29	36.7	228.8	40.23	NS
LDL (mg/dl)	118.70	26.15	134.6	30.00	NS
HDL (mg/dl)	48.43	10.14	43.56	10.79	NS
Hb (g/dl)	12.34	0.98	13.81	1.33	0.006
Plt (/ml)	$2.4x10^5$	$0.78x10^5$	$2.9x10^5$	$0.93x10^5$	NS

AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. ALP: Alkaline phosphatase. FBS: Fasting blood sugar. TG: Triglyceride. LDL: Low-density lipoprotein cholesterol. HDL: High-density lipoprotein cholesterol. Hb: Hemoglobin. Plt: Platelet.
[1] Mann-Whitney test, P value non-significant (NS) SD: Standard deviation.

predominance but more recent data suggest an equal to slight male predominance (9).

The overall prevalence of CD was 2.2% (7/316) in patients with NAFLD; however, it was 5.8% in patients with BMI value <27 kg/m^2. This rate is higher compared to the prevalence of 1/164 in the general population in Iran (4). Several studies have been carried out to estimate the prevalence of CD in a series of patients with unexplained liver damage; however, only a few have been focused on FLD as a subgroup of patients. In the study by Lo Iacono et al. (10), CD was diagnosed in 3.3% of NAFLD patients, but they did not specify anthropometric measurements and only patients with BMI values <30 kg/m^2 were included (10). Associations between obesity, type 2 diabetes mellitus and fatty liver have long been recognized. Many individuals labeled as 'non-obese' NAFLD (on the basis of BMI) have central obesity, and central obesity has strong correlations with insulin resistance and is a key feature of the metabolic syndrome (11-13). Obesity in the Asian population is defined as BMI ≥25 kg/m^2 (7). However, some patients in our study had normal weight and waist circumference (15.5% and 17.7%, respectively), so screening etiologies other than metabolic syndrome are necessary. Our data confirm the importance of CD screening when assessing subjects with NAFLD without an obvious metabolic syndrome. Indeed, a proportion of subjects with NAFLD had long-standing liver damage due to CD.

Our data substantiate the fact that not all patients with CD have normal or low BMI or waist circumference (14), as 2 of 7 patients (29%) had BMI values >25 kg/m^2 and 3 (43%) had abnormal waist circumference (Table 2).

Transaminase levels between CD and non-CD patients did not show a significant difference, but hemoglobin level in patients with CD was significantly lower than in non-CD individuals. This can be explained with enteropathy and malabsorption, although subclinical presentation in CD with liver involvement is more common (15,16); thus, CD screening in NAFLD patients associated with low or borderline hemoglobin levels is strongly recommended. The mean triglyceride level in patients with CD was >150 mg/dl, but significantly lower compared to non-CD patients. This can be explained by the higher frequency of obesity-related risk factors in non-CD patients.

EmA is a good marker of CD, but it is an expensive test. Human tTG ELISA test is similar to the EmA assay, with regard to sensitivity and specificity, and therefore is best suited for screening assays (17,18). However, in chronic liver disease of various etiologies, in connective tissue diseases and in inflammatory bowel diseases, a high rate of anti-tTG-false positives is found by using guinea pig or human recombinant tTG (19,20). In our study, one of the patients with positive serology for anti-tTG did not have histopathologic-proven CD in the duodenum, so the false-positive rate of IgA anti-tTG was 12.5% (confidence interval [CI]: 7.5%-17.5%). Some authors have demonstrated that the human tTG-based ELISA represents a cost-effective strategy for identifying both symptomatic and atypical forms of CD (21), but in chronic liver disease, this issue needs to be clarified. If available, HLA DQ2

and HLA DQ8 negativity in the setting of chronic liver disease almost exclusively rules out CD.

The pathophysiological mechanisms of liver damage in patients with CD are poorly understood. A widely accepted hypothesis is based on the fact that increased bowel permeability as seen in CD patients may favor antigen absorption from the intestine via the portal circulation (22). It has been demonstrated recently that NAFLD is associated with increased intestinal permeability and small intestinal bacterial overgrowth and that these factors are associated with the severity of hepatic steatosis. These data may suggest that the increased intestinal permeability may be the condition *sine qua non* for the hypothesis of the contribution of the gut–liver axis to the development of NAFLD (23).

In conclusion, the frequency of CD associated with NAFLD was significantly higher than rates reported in the general population in Iran. We suggest investigation of CD in these patients, especially in those with BMI value <27 kg/m^2 and/or anemia.

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