

Serologic testing for celiac disease in young people with elevated transaminases

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Background/aim: A cryptogenic elevation of transaminases is the most common hepatic manifestation in celiac disease (CD). In adult patients and pediatric patients with cryptogenic hypertransaminasemia, the prevalence of CD was 4% and 12%, respectively. However, there are no related data from China in this regard. We aimed to investigate the status of CD in young Chinese patients with elevated transaminases.

Materials and methods: A total of 125 patients with elevated transaminases and 125 healthy individuals as controls with matched age and sex were involved in the study. Serum markers of hepatitis B were determined in patients with elevated transaminases. All subjects were screened for CD by testing serum IgA antitissue transglutaminase antibodies (anti-tTG IgA), and total serum IgA was determined in order to rule out IgA deficiency.

Results: None of the subjects were seropositive to IgA anti-tTG antibodies. No association between CD and elevated transaminases was found. Hepatitis B viral infections were one of the main causes of raised transaminases.

Conclusion: Before the exclusion of every known cause of raised transaminase levels, routine serological screening for CD should not be recommended for patients who only present elevated transaminases.

Key words: Celiac disease, alanine aminotransferase, aspartate aminotransferases, hepatitis B virus

1. Introduction

Celiac disease (CD) is a T cell-mediated autoimmune enteropathy induced by the ingestion of gluten proteins, which exist in grains of wheat, barley, and rye (1). Gluten can trigger innate and adaptive immunity, which leads to intestinal epithelial cell impairment, increased mucosal permeability, villous atrophy, and nutrient malabsorption (2). Perennial malnutrition can further affect the functions of other body organs, leading to multisystem disorder. Liver impairment is one of the common extraintestinal manifestations of CD, and a wide variety of liver dysfunctions may occur with celiac disease (3). Among the liver disorders related to CD, a cryptogenic elevation of aminotransferases is the most common symptom, which is an early sign and the only sign in some patients with CD (3,4). Cryptogenic hypertransaminasemia had been reported in 27% of adults with CD and in 36% of children with CD, and in adult patients and pediatric patients with cryptogenic hypertransaminasemia, the

prevalence of CD was 4% and 12%, respectively (5,6). Elevated transaminase levels reverted to normal in the majority of patients on a gluten free diet (4–6). Therefore, routine screening for CD is recommended in patients with elevated aminotransferases of unknown origin, and it is also considered to be necessary among patients with abnormal liver function tests (7).

CD has been recently become a public health issue in many areas of the world, especially in Europe and the United States (8). CD was considered to be rare in China (9), and it has not been studied thoroughly, despite the fact that China is the largest wheat producer and wheat customer in the world. The consumption of wheat and other gluten-containing products is increasing rapidly in China, which carries concomitant risks for the development of CD. Based on the increased exposure to gluten and the predisposing HLA allele frequencies in the Chinese population, CD might be more common in China than currently reported (10). Moreover, previous studies

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about screening CD in at-risk groups in China found that the prevalence of CD was 6.5%–11.9% in patients with chronic diarrhea and 1.77%–8.2% in patients with diarrhea-predominant irritable bowel syndrome (11–14). However, there are no related data from China on the occurrence of CD in patients with elevated transaminases. Volta et al. reported that elevated transaminases might be early manifestations in young patients (mean age: 23 years) with asymptomatic CD (4). Therefore, we aimed to evaluate the necessity of screening for CD in young patients with elevated transaminases in this study.

2. Materials and methods

2.1. Patients and healthy controls

Subjects with elevated transaminases and controls were recruited from students who underwent routine physical examinations at the School Hospital of Nanchang Hangkong University, Nanchang, China from September 2010 to October 2010. The physical examination records were collected and investigated. A total of 125 patients with abnormal liver function tests were enrolled in the study; all their other physical examination results, including pulse rate, kidney function tests, and chest X-ray exam, were normal. As a control group, 125 unrelated healthy individuals with matched age and sex were also involved. Heights and weights of all subjects were collected from their physical examinations records and used to calculate body mass index (BMI) as kg/m^2 . BMI values of less than 18.5, between 24 and 27.9, and above 28 are considered as underweight, overweight, and obese (15), respectively.

All individuals recruited in this study were unrelated Han Chinese. The study was approved by the institutional ethics committee, and all subjects gave informed consent before participation.

2.2. Serological test

Blood samples (5 mL) were collected from all subjects after at least 10 h of overnight fasting and the avoidance of excessive exercise and fatigue, alcohol intake, and use of potential hepatic toxic medications before blood sampling. After centrifugation at $2130 \times g$ for 5 min, serum samples were obtained and stored at -80°C until use. The serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined again for the patients with a chemistry analyzer (BS300 chemistry analyzer, Mindray Medical International Ltd., Shenzhen, China). The upper limit of normal (ULN) of ALT was less than 31 IU/L and less than 41 IU/L for females and males, respectively, while the ULN of AST was <31 IU/L for females and <37 IU/L for males. Patients with elevated ALT and/or AST were tested for hepatitis B surface antigen (HBsAg), antibodies to hepatitis B surface antigen

(anti-HBs), hepatitis B e antigen (HBeAg), antibodies to hepatitis B e antigen (anti-HBe), and immunoglobulin G and immunoglobulin M antibodies to hepatitis B core antigen (IgG and IgM anti-HBc) by enzyme immunoassay (Autobio Diagnostics Co., Zhengzhou, China).

All of the 250 subjects were screened for CD by testing serum IgA anti-tissue transglutaminase antibodies (anti-tTG IgA) with a commercial QUANTA Lite h-tTG IgA ELISA kit (INOVA Diagnostics, San Diego, CA, USA). According to the recommendations of the manufacturer, tTG IgA antibody levels of <20 U were regarded as negative, 20 to 30 U as weak positive, and >30 U as moderate to strong positive. All samples were determined in accordance with the manufacturer's instructions, and the total amount of serum IgA was quantified by rate nephelometry on the IMMAGE 800 immunochemistry system (Beckman Coulter, Brea, CA, USA) in order to rule out IgA deficiency.

2.3. Statistical analysis

Data were analyzed using STATA statistical software (version 11.2, Stata Corp, College Station, TX, USA). Testing for normality was performed by Shapiro–Wilk test. Data were expressed as means and standard deviations or as median and interquartile range (IQR) for continuous variables as appropriate. For categorical variables, results were expressed as absolute numbers and percentages. Significance tests were performed using Student's t-test or the Mann–Whitney test for qualitative variables. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

3. Results

A total of 250 subjects were included in this study, including 125 subjects with elevated liver transaminases and 125 healthy subjects. Their characteristics are shown in Table 1. Subjects with elevated transaminases had higher BMIs than the controls, and 47.2% were overweight or obese ($\text{BMI} \geq 24 \text{ kg}/\text{m}^2$). However, none of the subjects were seropositive to IgA anti-tTG antibodies, and the level of IgA anti-tTG had no significant difference between the groups. In all subjects, total serum IgA level was normal without selective IgA deficiency.

Among the 125 subjects with elevated transaminases, 99 (79.2%) had increased levels of ALT and AST, while 26 (20.8%) presented only elevated ALT. Moreover, as shown in Table 2, a majority of the subjects had mild elevation of transaminases that were less than 5 times the ULN, and the AST/ALT ratio was under 1.0 (median: 0.56, IQR: 0.49–0.72). This indicated that hepatitis B viral (HBV) infections were one of the main causes of abnormal liver enzymes since 45 subjects (36%) were HBsAg- and/or HBeAg-positive (Table 3).

Table 1. Demographic and serological test data of elevated transaminases group and control group.

Characteristics	Elevated transaminases (n = 125)	Controls (n = 125)	P-value
Female/male	13/112	13/112	
Age, mean \pm SD (range), years	19 \pm 1 (17–21)	19 \pm 1 (17–23)	0.908
ALT, median (IQR), U/L	90.6 (61–129.7)	18.3 (12.7–23.9)	<0.0001
AST, median (IQR), U/L	51 (38–69.5)	24.3 (21.3–28.3)	<0.0001
Total IgA, median (IQR), mg/dL	216 (170–270)	194 (147–265)	0.089
Anti-tTG IgA, median (IQR), U	4.03 (3.29–5.19)	4.08 (3.6–5.47)	0.130
Positive anti-tTG IgA, n (%)	0 (0)	0 (0)	
BMI, median (IQR), kg/m ²	23.4 (20.3–27.7)	19.4 (18.6–21.2)	<0.0001
Underweight (<18.5), n (%)	12 (9.6)	29 (23.2)	
Normal (18.5–23.9), n (%)	54 (43.2)	87 (69.6)	
Overweight (24–27.9), n (%)	29 (23.2)	9 (7.2)	
Obese (\geq 28), n (%)	30 (24.0)	0 (0)	

BMI, Body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; anti-tTG IgA, immunoglobulin A antitissue transglutaminase antibodies (cutoff value: >20 U); SD, standard deviation; IQR, interquartile range.

Table 2. Magnitude of transaminase alterations in elevated transaminases group.

Magnitude of transaminases alteration	Patients with elevated ALT ^a (n = 125) n (%)	Patients with elevated AST ^b (n = 99) n (%)
<5 \times ULN	109 (87.2)	96 (97.0)
5–10 \times ULN	15 (12.0)	3 (3.0)
>10 \times ULN	1 (0.8)	0 (0)

ALT, Alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

^aULN: females 31 U/L, males 41 U/L.

^bULN: females 31 U/L, males 37 U/L.

4. Discussion

Liver function tests are routinely performed for primary care and diagnosis of liver disease, and ALT and AST are sensitive markers for hepatic cell damage. Their amounts can be increased in a spectrum of hepatic dysfunctions including alcohol-related liver damage, hemochromatosis, hepatitis B and C, nonalcoholic fatty liver disease (NAFLD), autoimmune hepatitis, Wilson's disease, and α_1 -antitrypsin deficiency. In addition to hepatic reasons, nonhepatic causes, e.g., CD, muscular disorders, and thyroid disorders, can also elevate the levels of transaminase (17,18).

The association between CD and various liver diseases has been confirmed, including cryptogenic hypertransaminasemia, autoimmune liver disease

(autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cirrhosis), NAFLD, and nonalcoholic steatohepatitis (3). However, it is still a controversy as to whether viral hepatitis (HBV and HBC) is associated with CD (19–22).

In this work, none of patients with elevated transaminases were positive for serum markers of CD (IgA anti-tTG antibody), and the association between CD and elevated transaminases was not addressed. Our finding is inconsistent with the previously reported studies. The most possible explanation for the inconsistency might be the different criteria of subject selection in different studies (4,7,23–25), since the subjects recruited for the evaluation of the relationship between CD and raised AST/ALT were

Table 3. The results of hepatitis B serologic tests in elevated transaminases group.

Results of hepatitis B serologic tests					n (%)	Interpretation (16)
HBsAg	anti-HBs	HBeAg	anti-HBe	anti-HBc (IgG+IgM)		
-	-	-	-	-	29 (23.2)	Uninfected and not immune, susceptible
-	+	-	-	+	21 (16.8)	Previously infected and immune due to natural infection
-	+	-	-	-	11 (8.8)	Clinical recovery, immune due to HBV vaccination
-	+	-	+	+	7 (5.6)	Recovered from HBV infection
-	-	-	-	+	12 (9.6)	Acute window period, past infection
+	-	-	-	-	4 (3.2)	Early acute HBV infection, chronic HBV carrier
+	-	-	-	+	1 (0.8)	Acute HBV infection, chronic HBV carrier
+	-	+	-	+	26 (18.4)	Acute HBV infection, chronic active infection, high infectivity
+	-	-	+	+	14 (11.2)	Convalescence, lower infectivity

+, positive; -, negative

HBsAg, Hepatitis B surface antigen; anti-HBs, antibodies to hepatitis B surface antigen; HBeAg, hepatitis B e antigen; anti-HBe, antibodies to hepatitis B e antigen; anti-HBc, antibodies to hepatitis B core antigen; HBV, hepatitis B virus; IgG, immunoglobulin G; IgM, immunoglobulin M.

classified as having unexplained elevated transaminase after the exclusion of every known cause of raised transaminase levels, e.g., alcohol or drug use, viral hepatitis, metabolic hepatic disease, and autoimmune liver diseases (4,23–25). Only a study conducted in Iran studied the necessity of routine screening for CD in patients with abnormal liver function tests irrespective of the reason for abnormal liver function tests (7), and 6 patients were diagnosed with CD among 224 patients with abnormal liver function. Those 6 patients had hypertransaminasemia, autoimmune hepatitis, and cryptogenic cirrhosis, respectively (7).

In the case of interference, alcohol, medications, and strenuous exercise, which can affect the level of transaminases, were first ruled out by inquiring. Moreover, since the prevalence of HBsAg is 7.2% in China (26), and HBV infection is one of most common causes of elevated transaminases, we performed further hepatitis B serologic tests in the patients with elevated transaminases. The test showed that 45 patients (36%) carried HbsAg, and this indicated that the main cause of elevated transaminases in these patients was HBV infection. However, the previous reported studies showed obscure associations between HBV and CD. Two groups from southern Iran (19) and Brazil (20) showed that the prevalence of definite CD by

duodenal biopsy in HBV patients was 5.4% and 9.1%, respectively, suggesting the necessity of screening for CD in patients with HBV. On the contrary, information from both Italy (21) and the Czech Republic (22) showed that none of the patients with HBV were diagnosed with CD, which was consistent with our findings. In addition, the majority of patients had mildly elevated transaminases levels and AST/ALT ratios of less than 1, and 24% of patients were obese in the current study. As we know, metabolic syndrome and obesity are correlated with NAFLD, and NAFLD cases also have mildly elevated ALT and AST with low AST/ALT ratios (<1) (27). NAFLD and metabolic syndrome were two main causes of abnormal liver tests among the Chinese population (28). Therefore, the most probable cause of elevated transaminases is NAFLD or metabolic syndrome in the patients recruited in this study, in addition to HBV infection.

The small sample size of the study could lead to bias of the results. In particular, the female-to-male ratio is low at only about 1:10, because previous studies had identified that the occurrence of CD in women is 2 to 3 times higher than in men (8,29,30). Obviously, we cannot claim that elevated transaminases have nothing to do with CD, although none of the patients with elevated transaminases

were seropositive for CD in this work. However, we think that serological screening for CD in a routine way should not be recommended for patients who only present elevated transaminases. Patients with only elevated transaminases need to be followed and reevaluated, and the identification of causes leading to elevated ALT and/or AST is very important if persistent transaminases increase. Screening for CD is recommended only for patients with unexplained elevated transaminase.

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