

Should we Test Patients with Chronic Dysphagia for Enterohepatic *Helicobacter* Infection?

Artsiom V Tsyrukunou, Timothy C Counihan, David Albert, Rangan Murali

Artsiom V Tsyrukunou, Department of Infectious Disease and Immunology, Infectious Disease Fellow, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, the United States

Timothy C Counihan, Department of Surgery, Berkshire Medical Center, 725 North Street, Pittsfield, MA 01201, the United States

David Albert, Internal Medicine Residency Program, Program Director, Berkshire Medical Center, 725 North Street, Pittsfield, MA 01201, the United States

Rangan Murali, Department of Gastroenterology, Berkshire Medical Center, 725 North Street, Pittsfield, MA 01201, the United States

Correspondence to: Artsiom V Tsyrukunou, Department of Infectious Disease and Immunology, Infectious Disease Fellow, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, the United States. artsiom.tsyrukunou@umassmemorial.org
Telephone: +1-508-856-3158 Fax: +1-508-856-5981

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ABSTRACT

AIM: It is recognized that *Helicobacter* species cause disease in the stomach and duodenum, however, the extent to which the enterohepatic cluster of this group of microorganisms may cause hepatobiliary disease is unknown. We aimed to explore the association between positive titers of anti-*Helicobacter pylori* immunoglobulin G, alanine aminotransferase levels and findings on upper endoscopy in patients with dyspeptic symptoms.

MATERIAL AND METHODS: Medical records review of 408 patients from outpatient clinics with dyspeptic symptoms was performed. All patients had no history of liver disease. Liver function tests, serological tests including quantitative anti-*Helicobacter pylori* with a threshold of 1.1 units/dL as a positive test, and endoscopy reports were reviewed up to 3 years prior to the *Helicobacter* antibody testing. Variables were created for each 3-month period preceding serologic test for *Helicobacter* infection. Statistical analysis using *t*-test, chi-square and Pearson correlation coefficients was performed.

RESULTS: Patients who tested positive for *Helicobacter pylori* antibodies were more likely to have elevated alanine aminotransferase

levels than patients with negative anti-*Helicobacter pylori* antibodies (10.5% vs 4.1%, $p < 0.05$). Many patients with positive anti-*Helicobacter pylori* antibodies had unexpectedly low rate of gastric invasion by *Helicobacter pylori*, but noted to have a high rate of alanine aminotransferase elevation (34.3%). In contrast, patients with positive serology for *Helicobacter pylori* infection and positive mucosal biopsy for *Helicobacter* invasion had a lower rate of alanine aminotransferase elevation (15.4%). A correlation between elevated alanine aminotransferase levels and the anti-*Helicobacter pylori* antibody titers ($r = 0.17$; $p < 0.01$) was observed.

CONCLUSION: These findings indicate that positive serological test for *Helicobacter pylori* infection may not be fully explained by invasion of gastric or duodenal mucosa alone. These data support the theory that *Helicobacter* species other than *Helicobacter pylori* may play a role in hepatobiliary diseases in humans.

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Key words: *Helicobacter* infection; Enterohepatic *Helicobacter* species; Anti-*Helicobacter* immunoglobulins; Alanine aminotransferase

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INTRODUCTION

The *Helicobacter* genus comprises over 32 species^[1]. It is recognized that *Helicobacter* species cause disease in the stomach and duodenum, however, the extent to which this group of microorganisms may cause human disease in other organ systems is unknown.

Enterohepatic *Helicobacters* have only recently been classified by investigators at the National Cancer Institute^[2]. Phylogenetically, *Helicobacter* species are divided into gastric and enterohepatic lineages^[3]. The enterohepatic cluster included *Helicobacter* hepaticus, *Helicobacter pullorum*, *Helicobacter canis*, *Helicobacter cholecystus*, *Helicobacter pametensis*, *Helicobacter cinaedi*, and

*Helicobacter mustelae*³. Many of the enterohepatic *Helicobacter* species are known to survive in hepatic bile ducts and liver of laboratory rodents²¹. These same species have been linked to chronic hepatitis, pancreatitis, and cholangiofibrosis^{4,51} in rodent models. In humans, the association of non-gastric *Helicobacter* species and hepatobiliary or intestinal diseases is not clear. One of the first non-gastric *Helicobacters* associated with proctocolitis was *Helicobacter cinaedi*^{16,71}. Bile samples taken from the gallbladders of patients with cholecystitis have been shown to be polymerase chain reaction positive for *Helicobacter bilis* DNA¹⁸¹. However, enterohepatic *Helicobacter* species have not been isolated in culture from either bile or the liver tissue¹⁸¹.

It has also been shown that antibody reactivity to cell surface protein of *Helicobacter hepaticus* and *Helicobacter bilis* was significantly higher in patients with chronic liver disease^{9,101}. The level of anti-*Helicobacter hepaticus* serum antibodies are particularly elevated in patients with liver cirrhosis comparing to chronic viral hepatitis group¹¹¹. A high rate of cross-reactivity between cell surface proteins of *Helicobacter pylori* and enterohepatic *Helicobacters*, such as *Helicobacter pullorum*, *Helicobacter bilis*, *Helicobacter hepaticus*¹⁹¹ has been demonstrated. It remains unclear whether titers of specific anti-*Helicobacter pylori* antibodies would be elevated in patients without gastritis or peptic ulcer disease but with evidence of extrahepatic *Helicobacter* DNA in the liver or bile.

The aim of this study was to review trends in hepatic transaminases levels over time in patients with chronic dyspeptic symptoms who were tested positive for anti-*Helicobacter pylori* antibodies, but have never been treated for *Helicobacter* infection prior to the serological test and who had subsequent upper endoscopy.

METHODS

The medical records of 552 adult patients who presented with dyspeptic symptoms to outpatient clinics and gastroenterology offices affiliated with Berkshire Medical Center, Pittsfield, MA were reviewed. Eligibility criteria are shown in figure 1.

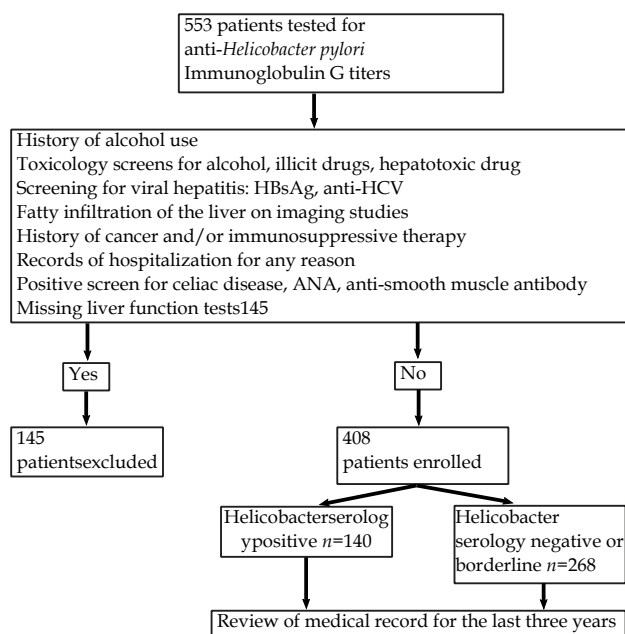


Figure 1 Eligibility criteria and screening methods for chronic liver disease. All study patients were free of any identifiable liver disease based on review of outpatient records. Negative titers for *Helicobacter* infection were 0-0.7 U/mL. Borderline titers were 0.8-1.0 U/mL.

Patients enrolled into the study had no history of alcohol use, viral hepatitis, fatty liver disease, cancer or were taking medications which could cause hepatotoxicity. The information was reviewed for three consecutive years. Separate variables were analyzed for each 3-month period preceding serologic test for *Helicobacter* infection. Standard FDA approved ELISA kit Immulite 2000 for *Helicobacter pylori* immunoglobulin G detection was used with threshold of 1.1U/dL as a positive test with 3 levels of control from Diagnostic Products Corporation (DPC), Los Angeles, CA, USA. Clinical and laboratory data including liver function tests, serological tests, abdominal imaging and endoscopy reports were reviewed up to 3 years prior to the *Helicobacter* antibody testing to see whether elevation of anti-*Helicobacter* antibody titers was associated with any changes in the liver function tests. Interlaboratory differences in measuring alanine aminotransferase levels were mitigated by using the percentage from the top referenced level at a particular laboratory. Metabolic variability in the levels of hepatic transaminases was assessed by glycolated hemoglobin and thyroid stimulating hormone levels. Statistical analysis was performed on SAS Institute 9.1 software using t-test, chi-square and Pearson correlation coefficients to explore the relationship between levels of alanine aminotransferase and levels of specific anti-*Helicobacter pylori* antibodies. The study was approved by the Institutional Review Board of the hospital.

RESULTS

Patients were seen for evaluation of dyspepsia at their primary care physicians' office for testing including basic blood tests with liver function panel, right upper quadrant ultrasound, and upper endoscopy. Unclear etiology of dyspepsia led to an additional workup which included anti-*Helicobacter* immunoglobulin G testing.

Patients with positive serology for *Helicobacter pylori* infection were more likely to have abnormal levels of alanine aminotransferase during the study period (Table 1).

Table 1 Frequency of abnormal alanine aminotransferase levels in patients without underlying liver disease with chronic dyspeptic symptoms.

	Patients with positive serology for <i>Helicobacter pylori</i> infection	Patient with negative serology for <i>Helicobacter pylori</i> infection	P Value
Number of patients with abnormal alanine aminotransferase levels, (n, percent)	9/86 (10.4%)	7/170 (4.1%)	0.047

Patients with positive anti-*Helicobacter pylori* antibodies had higher number of male patients 61 (43.6%) comparing to the group who tested negative for anti-*Helicobacter pylori* antibodies 71 (26.5%). An analysis controlling for gender and levels of glycolated hemoglobin was performed. There were no differences noted in levels of alanine aminotransferase in either gender group or both high and low glycolated hemoglobin level groups. The average levels of glycolated hemoglobin were 6.2% and 5.8% in *Helicobacter pylori* positive group and *Helicobacter pylori* negative group, respectively. The levels of thyroid stimulating hormone were 2.3 μ U/mL in *Helicobacter* positive patients and 2.1 μ U/mL in *Helicobacter* negative group.

It was noted that several age groups had transient elevation of alanine aminotransferase levels before the confirmation of *Helicobacter pylori* infection was made by serological tests. When patients were divided in arbitrary age groups, elevation of liver transaminases was seen in age group over 37 years (Table 2). It was noted again in 37-54 age group at the time of the anti-*Helicobacter* antibody testing and 3 months prior to the test. *Helicobacter pylori* positive patients with

Table 2 Levels of alanine aminotransferase in patients within three consecutive years prior to dyspeptic symptoms and testing for specific anti-*Helicobacter pylori* antibodies by age.

	18-36 years old			37-54 years old			55-72 years old			Over 72 years old		
	HP-	HP+	P Value	HP-	HP+	P Value	HP-	HP+	P Value	HP-	HP+	P Value
Same day with anti- <i>Helicobacter pylori</i> testing	55.9±42.4	69.0±55.7	0.29	49.4±18.8 n=73	69.9±47.3 n=21	0.003	55.0±21.0	55.7±23.7	0.90	55.0±21.0	55.7±23.7	0.90
Within 0-3 months	44.7±33.7	71.8±38.9	0.19	53.9±19.7 n=32	91.0±61.6 n=5	0.01	50.9±26.3	58.5±19.9	0.35	50.9±26.3	58.5±19.9	0.35
Within 4-6 months	53.7±37.5	51.9±12.7	0.91	55.4±40.3	70.9±54.7	0.40	45.9±11.3 n=16	64.1±26.8 n=14	0.02	45.9±11.3 n=16	64.1±26.8 n=14	0.02
Within 7-9 months	42.9±11.6	43.0±16.9	0.98	56.7±49.0	59.7±26.2	0.85	47.4±14.1 n=23	60.1±24.0 n=19	0.03	47.4±14.1 n=23	60.1±24.0 n=19	0.03
Within 10-12 months	52.2±52.4	81.1±66.1	0.46	56.7±35.0	67.2±25.6	0.47	50.6±22.2	62.2±28.5	0.23	50.6±22.2	62.2±28.5	0.23
Within 13-24 months	43.4±19.4	50.2±14.9	0.38	51.9±32.6	58.1±20.4	0.44	48.9±17.3 n=47	59.8±27.3 n=27	0.04	48.9±17.3 n=47	59.8±27.3 n=27	0.04
Within 25-36 months	49.1±33.4	40.5±20.1	0.63	44.2±22.7	44.7±8.9	0.97	52.0±26.2	64.2±23.9	0.15	52.0±26.2	64.2±23.9	0.15

Plus-minus values are means of alanine aminotransferase (percent of upper limit of normal value)±standard deviation; HP: *Helicobacter pylori*; "HP-": Negative titers of anti-*Helicobacter pylori* immunoglobulin G; "HP+": Positive titers of anti-*Helicobacter pylori* immunoglobulin G.

age of 55-72 had several episodes of statistically significant elevation liver transaminase within two years prior to anti-*Helicobacter pylori* antibodies test. Elevation of alanine aminotransferase was noted in *Helicobacter pylori* negative patients with age from 55 years to 72 years within two years prior to the serologic testing; however the sample size for this particular group was low.

Overall, patients with positive anti-*Helicobacter pylori* antibodies had higher levels of alanine aminotransferase comparing to patients

on the tissue biopsy. One third of the patients with negative mucosal biopsy for *Helicobacter pylori* despite positive *Helicobacter pylori* serology and no prior treatments had elevated liver transaminases. On the other hand, the abnormal alanine aminotransferase levels was seen only in 2 out of 13 patients who had positive smears for *Helicobacter pylori* in mucosa and all of them had inflammatory changes in the mucosa. Thus, there was an observation of abnormally high incidence of alanine aminotransferase in patients with normally appear-

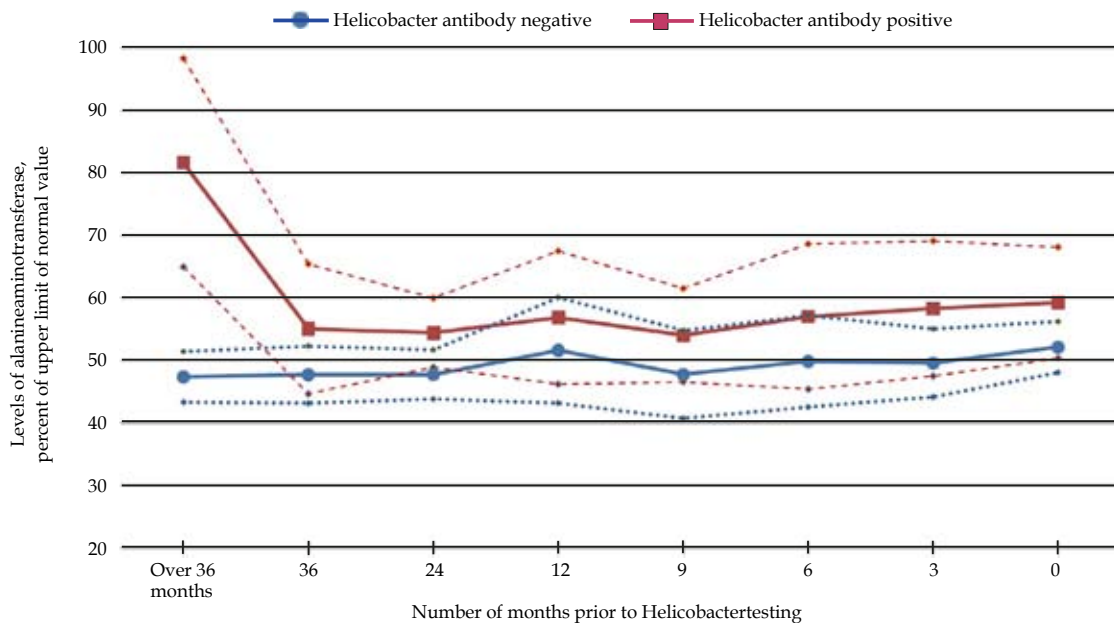


Figure 2 Average levels of alanine aminotransferase with 95% confidence intervals at each 3-month period preceding the testing for anti-*Helicobacter* antibodies.

with borderline and negative levels of specific antibodies. However the differences were not statistically significant at any time within the 3 year period prior to the test (Figure 2).

Patients with elevated titers of anti-*Helicobacter pylori* antibodies were selected for further review of upper endoscopy results to see whether positive serology was associated with inflammatory changes on biopsy and changes in liver transaminases. There were 48 patients who had both positive serology for *Helicobacter pylori* infection and endoscopy performed with biopsies. Alanine aminotransferase levels were reviewed within the last three years prior to serological test in this group of the patients (Table 3.). All patients who had evidence of *Helicobacter pylori* in the mucosa expectedly had inflammatory changes on mucosal biopsy. Only one half of the patients with negative microscopy for *Helicobacter pylori* had inflammatory changes

Table 3 Frequency of inflammatory changes in stomach and duodenal mucosa in patients with elevated alanine aminotransferase levels who tested positive for anti-*Helicobacter pylori* antibodies prior to *Helicobacter* treatment or endoscopy.

	Positive microscopy for <i>Helicobacter pylori</i> on biopsy, n	Negative microscopy for <i>Helicobacter pylori</i> on biopsy, n	P-Value ¹
Elevated alanine aminotransferase prior to endoscopy, (n, percent)	2 (15.4%)	12 (34.3%)	0.21
Always normal levels of alanine aminotransferase prior to endoscopy, (n, percent)	11 (84.6%)	23 (65.7%)	
Total number of patients, (n)	13	35	

¹p Value used to compare frequency of elevated alanine aminotransferase in patients with positive vs negative microscopy for *Helicobacter pylori* on mucosal biopsy.

ing gastric or duodenal mucosa, positive serology for *Helicobacter pylori* with documented absence of the microorganisms in the tissue.

Correlation analysis was performed to explore whether there was any relationship between the degree of elevation of anti-*Helicobacter* titers and levels of alanine aminotransferase. There was a very mild trend towards positive correlation noted which was not statistically significant (Figure 3A). Further exploration of alanine aminotransferase levels in studied patients prior to serological tests was performed using data from three consecutive years before the anti-*Helicobacter pylori* testing (Figure 3B). Analysis showed mild positive correlation

clinical practice may also detect antibodies against other species of *Helicobacter* genus, but this possibility is valid and was described^[9,11]. There is no convincing data that enterohepatic *Helicobacters* can cause pathologic process in the liver or even cause a transient elevation of hepatic transaminases despite findings of *Helicobacter* organisms in liver tissue and bile. However, based on our study there was no other explanation of transient elevation of alanine aminotransferase in carefully selected patients with unexplained dyspeptic symptoms.

In our study patients who tested positive for *Helicobacter pylori*

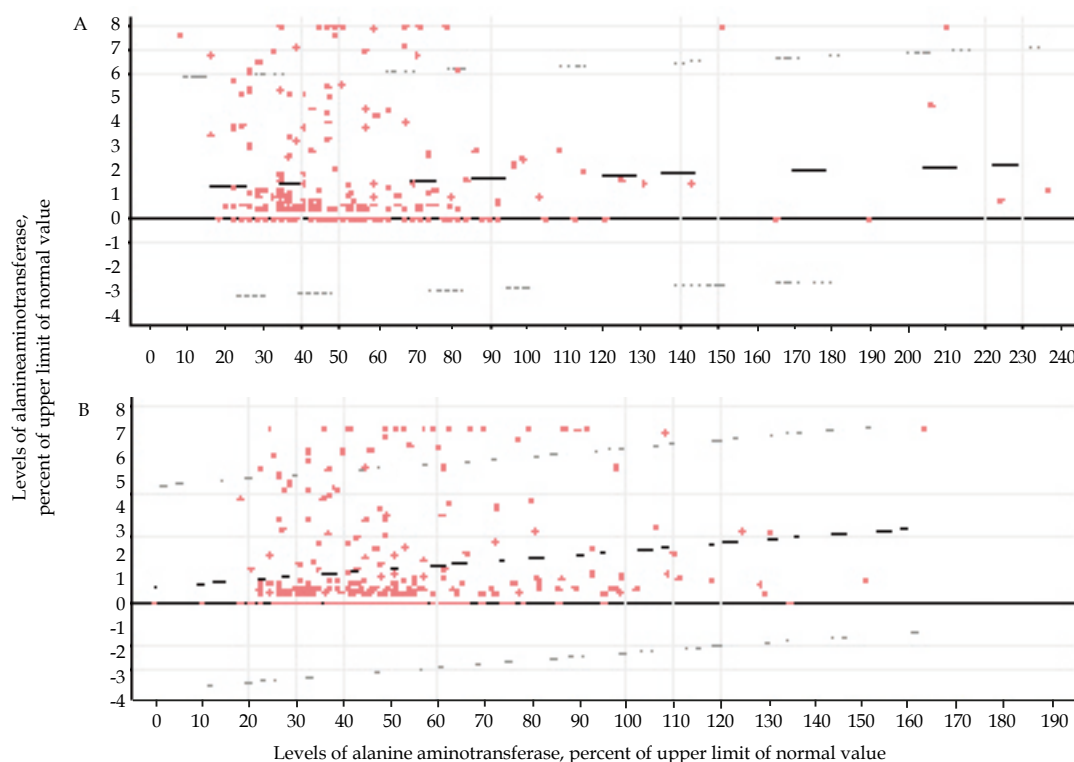


Figure 3 A: Correlation between levels of anti-*Helicobacter pylori* titers and alanine aminotransferase levels at the time of presentation for the serologic study. B: Retrospective analysis of levels of anti-*Helicobacter pylori* titers and alanine aminotransferase levels within the three consecutive years prior to the start of the symptoms which triggered anti-*Helicobacter pylori* antibody testing. Anti-*Helicobacter pylori* Immunoglobulin G titer of 1.1U/dL was diagnostic for *Helicobacter* infection.

($r=0.17$; $p<0.01$) between the alanine aminotransferase levels and the specific anti-*Helicobacter pylori* antibody titers.

DISCUSSION

Helicobacter species DNA has been found in the liver tissue and bile in humans^[8]. Currently, there is no doubt that newly recognized group of enterohepatic *Helicobacters* exists and affects laboratory animals. It is not clear whether hepatic *Helicobacters* are pathogenic in humans. *Helicobacter* species are known to cause diarrheal illnesses and affect patients with hepatocellular carcinoma, inflammatory bowel disease, and cholecystitis^[8-10]. Our study was performed on a population with a low incidence of *Helicobacter pylori* infection in the United States. However, all patients were symptomatic enough to trigger extensive evaluation by their primary care physicians and gastroenterologists, which could have increased pretest probability of the serological study. All patients were carefully pre-screened for possible hepatobiliary pathology and drug toxicities which could potentially affect the liver function test.

It is not clear whether serological tests designed for detection of *Helicobacter pylori* infection, which widely available for

antibodies were more likely to have elevated amine aminotransferase than patients with negative anti-*Helicobacter pylori* antibodies. It was shown that alanine aminotransferase levels are higher in patients who tested positive for *Helicobacter pylori* antibodies. Overall, the differences were not statistically significant at any time and elevation of hepatic transaminase was mild. After controlling for possible confounders it was found that older patients within a specific age group had elevated alanine aminotransferase within two years prior to the serologic test. The significance of this finding is not clear. The elevation of transaminases lasted only several months. It was assumed that patients tested positive for *Helicobacter pylori* infection could have started treatment and liver function tests after positive serology were not followed.

The patients with positive serology for *Helicobacter pylori* infection and negative smears for *Helicobacter pylori* in the stomach and duodenal mucosa tended to have higher rate of alanine aminotransferase elevation comparing to the group who had evidence of *Helicobacter* invasion on biopsy. All patients presented in the table 3 had positive *Helicobacter pylori* titers, which indicated active or recent *Helicobacter pylori* infection prior to the treatment. Patients with evidence of *Helicobacter pylori* invasion on tissue

biopsy expectedly had inflammatory changes in mucosa. However, patients with negative histology for *Helicobacter pylori* had a lower rate of mucosal inflammation and unexplained high rate of alanine aminotransferase elevation. This finding raises a possibility of ongoing *Helicobacter* infection other than *Helicobacter pylori* or at least an immunologic response to the presence of other *Helicobacter* species, which should be further explored.

When a patient presents to a provider with dyspeptic symptoms including epigastric pain, one of initial tests may include *Helicobacter pylori* antibody. If the test comes back as positive and patient does not have any alarming symptoms then most of the providers will employ a test-and-treat strategy for *Helicobacter* infection without performing endoscopy. Based on results of this study, there may be a group of patients who have positive serology for *Helicobacter pylori*; however they may not have evidence of active infectious process caused by *Helicobacter pylori* in the stomach or duodenum. Moreover, these patients may have more frequent elevation of alanine aminotransferase levels associated with positive serology which we cannot explain at this time. Additional studies with serological assays targeted directly to enterohepatic *Helicobacters* would be helpful to explore this immunologic phenomenon. Enterohepatic *Helicobacter*-specific antibodies could be analyzed after absorption of *Helicobacter pylori* antibodies or using monoclonal antibodies which are not widely available^[12].

There are several limitations to the study. Studied patients had relatively low pretest probability for the positive anti-*Helicobacter pylori* antibodies testing. However, all patients had unexplained dyspeptic symptoms and they were already tested for *Helicobacter* infection in the outpatient settings. Testing was clinically indicated due to suspicion of peptic ulcer disease. Unfortunately, specific anti-enterohepatic *Helicobacters* antibody assays are not widely available, which limited study for only anti-*Helicobacter pylori* antibody testing. There also might be other confounding factors, which could make influence on alanine aminotransferase levels, such as change in diet, exercise, weight, and body mass index. We used glycolated hemoglobin levels and thyroid stimulating hormone to detect obvious differences in the metabolic variability.

The correlation between elevated anti-*Helicobacter* titers and alanine aminotransferase levels indicates a possible association of *Helicobacter* infection and changes in liver function tests. Our data showed that the patients with negative endoscopy results for inflammation and the absence of *Helicobacter* microorganisms on tissue biopsy had a higher rate of alanine aminotransferase elevation. This may indicate that there may be transient elevation of liver function tests associated with dyspeptic symptoms which is not related to gastritis or duodenitis. However, it is not clear whether this elevation has a definite connection to different species of *Helicobacter* genus at this time. Additional studies to explore the role of *Helicobacter* species in hepatobiliary disease using highly specific anti-enterohepatic *Helicobacter* antibodies should be carried out in the future.

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Peer reviewer: Lucia Pacifico, MD, Department of Pediatrics, Sapienza University of Rome, Viale Regina Elena,324, 00161-Rome, Italy.