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Regular paper

Conflicting results of non-invasive methods for detection of *Helicobacter pylori* infection in children with celiac disease — a preliminary study

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Background: There are no data addressing the usefulness of non-invasive tests for the detection of *Helicobacter pylori* (HP) infection in celiac disease (CD). Aim: The aim of this study was to compare two most sensitive and specific tests — urea breath test (UBT) and fecal antigen test (FAT) in HP diagnosis in CD patients. Materials and Methods: The study comprised of 76 CD patients, 49 healthy subjects (HS) and 35 patients who underwent differential diagnosis due to abdominal pain (AP patients). The presence of HP infection was evaluated using the ^{13}C isotope-labeled UBT and FAT (ELISA). Results: HP infection was diagnosed based on UBT and FAT in 8 (16.3%) and 7 (14.3%) HS, and in 8 (10.5%) CD patients and 12 (34.3%) AP patients, respectively, using both tests. The prevalence of conflicting results in comparison with positive results (obtained with any of the two tests) was distinctly higher (54.5%) in CD group than in other subjects (23.3%); however, due to low HP prevalence, it did not reach the level of significance ($p<0.1759$). Conclusion: CD may increase the risk of divergent results of non-invasive tests used for the detection of HP infection in children. Since UBT is the most reliable test, we suggest its standard use as a method of choice in pediatric CD — at least until new evidence emerges supporting a different approach.

Key words: *Helicobacter pylori*, celiac disease, fecal test, breath test, urea

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INTRODUCTION

Helicobacter pylori (HP) is a first class carcinogen and definitely still belongs to a group of the most common human pathogens (Custers *et al.*, 2006). The relationship between HP infection and several gastrointestinal pathologies such as chronic gastritis, duodenal ulcer, and mucosa-associated lymphoid tissue lymphoma has been proved. Without any doubt HP colonization significantly multiplies the risk of gastric cancer (Ekstrom *et al.*, 2001).

Several tests are used for the detection of HP infection (Dzierżanowska-Fangrat *et al.*, 2006). Among the non-invasive diagnostic tests, urea breath test (UBT) and fecal antigen test (FAT) have definitely higher accuracy than serological or urinary antibody-based tests (Asaka *et al.*, 2010; Malfertheiner *et al.*, 2012). UBT has been considered to be the most reliable noninvasive test. However, its cost is relatively high. Furthermore, proton pump inhibitor (PPI) use may result in lower HP urease activity potentially leading to false-negative results in some cases (Malfertheiner *et al.*, 2012).

There are only few publications directly comparing the usefulness of UBT and FAT in pediatrics. Moreover, the obtained results are contradictory (Lahner *et al.*, 2004; Kato *et al.*, 2004; Shaikh *et al.*, 2005; Drzymala-Czyż *et al.*, 2014). Since there are no data for celiac disease (CD) in which potential effect of the disease on the results of both tests was studied, we aimed to assess their reliability for the diagnosis of HP infection in this clinical entity.

MATERIALS AND METHODS

The study presented here was a retrospective study comparing two non-invasive tests — UBT and FAT for diagnosis of HP infection in CD patients. The inclusion criteria comprised: newly diagnosed CD patients (Koletzko *et al.*, 2012) with positive histopathology and positive tissue transglutaminase and anti-endomysial antibodies, age 3 years and older. Exclusion criteria were intravenous/oral antibiotics or PPIs for four weeks prior to the investigation. The study population consisted of 76 CD subjects (35 males and 41 females) aged from 3 to 16 years, in whom the presence of HP was diagnosed routinely in the course of diagnostic procedures using both tests. The basic clinical characteristics of CD patients is given in Table 1.

The first control group consisted of 49 healthy subjects (HS) aged 4 to 19 years. The study was part of the project titled "PL0361/Good diagnosis - treatment-life" which was carried out by the Medical University Hospital in Zabrze (Poland) for evaluating the incidence of gastrointestinal diseases in Poland (Żabka *et al.*, 2010). The second control group included 35 patients aged 4 to 18 years, who underwent differential diagnosis due to abdominal pain (AP patients). No subject received intra-

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Abbreviations: AP, abdominal pain; CD, celiac disease; FAT, fecal antigen test; HP, *Helicobacter pylori;* HS, healthy subjects; PPI, proton pump inhibitor; UBT, urea breath test

Table 1. Clinical and demographic characteristics of CD patients

Clinical parameters	Mean ±S.D.
Age (years)	7.0±2.6
Sex: Males/females	35/41
Z-score for body height	–1.2±0.3
Z-score for body weight	–1.3±0.4

venous/oral antibiotics and PPIs for four weeks prior to the investigation.

The presence of HP was assessed in all subjects using the ^{13}C isotope-labeled UBT (IRIS, Wagner Analysen Technik, Bremen, Germany) and fecal test using a commercially available monoclonal antibody kit (Helicobacter Pylori Antigen ELISA Kit Diagnostic Automation, Calabasas, CA, USA). The tests were performed as described earlier (Drzymała-Czyż *et al*., 2013; Józefczuk *et al*., 2015).

Statistical methods. The difference in distribution of the HP status between groups was analyzed by the χ^2 test. *P* value < 0.05 was considered as statistically significant. All statistical analyses were performed using Statistica 9.0 software (StatSoft Inc., Tulsa, USA).

Ethical considerations. The protocol of the investigation was approved by the Ethical Committee of the Poznań University of Medical Sciences, Poland.

RESULTS

The number of positive HP test results detected by UBT and FAT in CD patients, HS and AP patients is presented in Table 2. In the group of 76 CD patients, HP infection was diagnosed in 8 (10.5%) based upon both, UBT and FAT. Among 49 HS and 35 AP patients, the infection was found in 8 (16.3%) and 12 (34.3%) based on UBT, and in 7 (14.3%) and 12 (34.3%) based on FAT, respectively. HP detection with the use of these two tests gave comparable results in all groups. Using UBT as the standard, the specificity of FAT in all three study groups was high. The sensitivity was lower and inconsistent (Table 3).

The contrasting results between the two tests in comparison with all results were found in 8.0% of cases in the CD group, in 6.1% in the control group and in 11.4% in the AP group (Table 4). Three CD patients, two HS and two AP patients were positive based on UBT and negative based on FAT, and three CD patients, one HS and two AP patients were negative based on UBT and positive based on FAT (Table 2). The prevalence of conflicting results in comparison with positive results (obtained in any of the two tests) was distinctly higher in the CD group (54.5%) than in other subjects (23.3%); however, due to low HP prevalence, it did not reach the level of significance ($p < 0.1759$; Table 4).

Table 3. The sensitivity and specificity of the fecal antigen test in comparison with the urea breath test in celiac disease patients (CD), healthy subjects (HS) and patients with abdominal pain (AP).

	CD	Others		
		HS	AP	Together
Sensitivity	5/8 (62.5%)	6/8 (75.0%)	10/12 (83.3%)	16/20 (80.0%)
Specificity	65/68 (95.6%)	40/41 (97.6%)	21/23 (91.3%)	61/64 (95.3%)

DISCUSSION

To our best knowledge, this is the first study directly comparing applicability of UBT and FAT for the detection of HP infection in CD pediatric patients. The high prevalence of discordant positive results (54.5%) in UBT and FAT was documented (Table 4). Concordant positive results in both tests were found in 5 out of 11 patients only. In 3 patients with positive results in FAT, the results of UBT were negative. Similarly, in 3 patients with positive results in UBT, the results of FAT were negative (Table 2).

According to available meta-analyses assessing UBT and FAT accuracy, the sensitivity and specificity of these tests are very high (Gisbert *et al*., 2006; Leal *et al*., 2011; Zhou *et al*., 2014; Ferwana *et al*., 2015). The overall sensitivity and specificity of ^{13}C UBT in adults were 96% and 94% (Ferwana *et al*., 2015), in children similar values were observed (95% and 94%, respectively) (Leal *et al*., 2011). The overall sensitivity and specificity of monoclonal FAT in adults were 93% and 96% (Gisbert *et al*., 2006), in children similar values were reported (96.2% and 94.7%, respectively) (Zhou *et al*., 2014). However, in many studies the methods chosen as a gold standard could rise some doubt (Gisbert *et al*., 2006; Leal *et al*., 2011; Zhou *et al*., 2014; Ferwana *et al*., 2015).

As compared with control groups, the percentage of CD patients with conflicting results between UBT and FAT in relation to all results (8.0%) was similar to that of HS (6.1%) and non-significantly lower than in AP patients (11.4%). Since the prevalence of HP infection significantly varied in groups studied here (higher in the AP group), we recalculated the obtained results in relation to positive results. The percentage of CD patients with

Table 2. The results of *Helicobacter pylori* (HP) detection based on the urea breath test (UBT) and the fecal test (FAT) in celiac disease patients (CD), healthy subjects (HS) and patients with abdominal pain (AP).

		CD		HS		AP	
Type of HP test		UBT					
	Results	positive n (%)	negative n (%)	positive n (%)	negative n (%)	positive n (%)	negative n (%)
FAT	Positive n (%)	5 (6.5%)	3 (4.0%)*	6 (12.3%)	1 (2.0%)*	10 (28.6%)	2 (5.7%)*
	Negative n (%)	3 (4.0%)*	65 (85.5%)	2 (4.1%)*	40 (81.6%)	2 (5.7%)*	21 (60.0%)

*Discordant results between UBT and FAT

Table 4. The prevalence of divergent *Helicobacter pylori* (HP) tests results in celiac disease patients (CD), healthy subjects (HS) and patients with abdominal pain (AP).

Conflicting results* in relation to	Others			
	CD	HS	AP	Together
All results	6/76 (8.0%)	3/49 (6.1%)	4/35 (11.4%)	7/84 (8.3%)
Positive results**	6/11 (54.5%)	3/9 (33.3%)	4/14 (28.6%)	7/23 (30.4%)

*+/– and –/+ HP test results in FAT and UBT; **positive result of at least one test (+/–, +/+, –/+)

conflicting results between UBT and FAT in relation to positive results (54.5%) was higher than in HS (33.3%) and in AP patients (28.6%). However, this difference has not reached statistical significance.

The percentage of discordant results in CD patients in the present study (8.0%) is considerably lower than in the majority of other studies (4.9–37.0%) (Masoero *et al.*, 2000; Lahner *et al.*, 2004; Kato *et al.*, 2004; Shaikh *et al.*, 2005; Drzymała-Czyż *et al.*, 2014). The prevalence of HP infection in CD patients and HS in the present study was also significantly lower (Table 5). Therefore, we recalculated conflicting values in relation to positive results. The percentage of conflicting results calculated in this way was higher in: adult patients treated due to HP infection (Masoero *et al.*, 2000), diagnosed with atrophic body gastritis (Lahner *et al.*, 2004); pediatric patients with malnutrition (Shaikh *et al.*, 2005), cystic fibrosis (Drzymała-Czyż *et al.*, 2014) and CD in the present study (44.3–60.0%) than in untreated (Masoero *et al.*, 2000) and pediatric (Kato *et al.*, 2004) populations with suspected gastrointestinal involvement (9.5–34.5%). However, the observed percentage was the highest among all pediatric populations studied so far.

UBT is regarded as the most reliable method (the gold standard) in diagnosing HP infection. FAT offers similarly high sensitivity and specificity. However, in some cases both tests may give false negative or false positive results. It seems likely that in case of concomitant diseases (like for CD in the present study) the concordance between UBT and FAT in detection of HP infection may be less tight. Having in mind the results obtained in the present study, the doubt appears whether the UBT and FAT are really equally reliable in the detection of HP infection in CD patients. It has been demonstrated that HP — besides its well-known spiral form — may exist in coccoid and degenerative forms which cannot be cultured. However, their antigens may be detected using FAT (Azevedo *et al.*, 2006; Andersen & Rasmusen, 2009; Casaola-Rodriquez *et al.*, 2013). On the other hand, UBT in such a case should be negative. Potential diagnostic divergence in CD may be related to several other underlying factors. Disturbed esophageal motility, altered gastric emptying and dysmotility of the small intestine, gallbladder and colon are common in untreated CD. Most of these motor abnormalities resolve after a strict gluten-free diet. However, some of them may persist (Pinto-Sanchez *et al.*, 2015).

Chronic superficial gastritis is several times more frequent in CD patients than the prevalence of HP infection. Moreover, lymphocytic gastritis has been reported in pediatric CD subjects (Nenna *et al.*, 2012). Potentially, it may influence the assessment of HP presence using UBT. On the other hand, the accuracy of FAT may be diminished when stool samples are more loose as may frequently happen in CD (Shimoyama, 2013).

Table 5. The prevalence of divergent *Helicobacter pylori* (HP) test results in different populations studied using urea breath test (UBT) and fecal test (FAT)

Study	*H. pylori* positive n (%)		Conflicting results* in relation to	
	UBT	FAT	All results n (%)	Positive results** n/N (%)
Masoero *et al.*, 2000 52 adults (untreated)	19 (36.5)	29 (55.8)	10 (19.2)	10/29 (34.5)
Masoero *et al.*, 2000 73 adults (treated)	21 (28.8)	42 (57.5)	27 (37.0)	27/45 (60.0)
Lahner *et al.*, 2004 27 adults (atrophic body gastritis)	8 (29.6)	4 (14.8)	4 (14.8)	4/8 (50.0)
Kato *et al.*, 2004 123 children suspected of HP	4 (3.3)	6 (4.9)	6 (4.9)	6/63 (9.5)
Shaikh *et al.*, 2005 86 asymptomatic children (malnourished)	45 (52.3)	50 (58.1)	27 (31.4)	27/61 (44.3)
Drzymała-Czyż *et al.*, 2014 79 children and young adults with cystic fibrosis	15 (19.0)	12 (15.2)	9 (11.4)	9/18 (50.0)
Present study 76 children with celiac disease	8 (10.5)	8 (10.5)	6 (8.0)	6/11 (54.5)
Present study 84 other subjects	20 (33.8)	19 (22.6)	7 (8.3)	7/23 (30.4)

*+/– and –/+ HP test results in FAT and UBT; **positive result of at least one test (+/–, +/+, –/+)

It seems that CD may increase the risk of divergent results of non-invasive tests used for the detection of HP infection in children. Since UBT is the most reliable test, we suggest its standard use as a method of choice in pediatric CD, at least until obtaining the reliable and valid results allowing for a change in such an approach. The major limitation of the present study is related to the relatively small number of HP infected CD patients included in the analysis. It results from the low prevalence of HP infection in the Polish pediatric population (Żabka *et al*., 2010). Although the statistical power of the study does not allow to draw unequivocal clinical conclusions, the question of whether UBT and FAT are equally reliable in the terms of HP detection in CD patients still remains current and requires further investigation.

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