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Non-invasive detection of *Helicobacter pylori* in cystic fibrosis – The fecal test vs. the urea breath test

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Abstract. – OBJECTIVES: Only recently it has been proven that cystic fibrosis (CF) patients have the same prevalence of Helicobacter pylori (HP infection) as the general population, as well as the same spectrum of changes caused by this pathogen. The aim of this study was to assess the reliability of the two most popular noninvasive tests – the urea breath test (UBT) and the fecal test (FT) in diagnosing HP infection in CF patients.

PATIENTS AND METHODS: The study was conducted on 79 CF patients and 49 healthy subjects (HS). The presence of HP infection was evaluated using the 13C isotope-labeled urea breath test and the fecal test (ELISA).

RESULTS: Fifteen (19.0%) CF patients and eight (16.3%) HS were found to be HP positive using the UBT. The HP stool antigen was detected in twelve (15.2%) CF patients and seven (14.3%) HS. Discordant results for the two tests were obtained in 9 out of 18 (50.0%) CF patients and 3 out of 9 (33.3%) HS. Although the differences were not statistically significant, the risk of potentially false negative and false positive results in CF subjects seems to be high. Similarly, no statistical differences in the basic clinical parameters were documented between the CF subgroups with concordant and divergent HP results.

CONCLUSIONS: Since there is convincing evidence of divergent UBT and FT results in the CF patients, we suggest that UBT is kept as the standard method for HP detection in this population, at least until obtaining reliable and valid results allows for a change in such an approach.

Key Words:
Helicobacter pylori, Cystic fibrosis, Fecal test, Urea breath test.

Abbreviations

CF = cystic fibrosis; HP = *Helicobacter Pylori*; UBT = urea breath test; FT = fecal test; HS = healthy subjects; PPI = proton pump inhibitors; ALT = alanine aminotransferase; AST = aspartate aminotransferase; INR = International Normalized Ratio; PA = *Pseudomonas aeruginosa.*

Introduction

Helicobacter pylori (HP) is the first formally recognized bacterial carcinogen, and is one of the most successful human pathogens, as over 50% of the world's population is colonized with this Gram-negative bacterium[1]. The risk factors for HP infection include low socioeconomic status, household crowding, ethnicity, migration from high prevalence regions, and infection status of family members[2]. In the absence of antibiotic therapy, the infection may persist for years and possibly even for an entire life if acquired in childhood[3]. Widely accepted is the relationship of HP colonization with the occurrence of the gastrointestinal pathologies such as chronic gastritis, duodenal ulcer, gastric ulcer, and mucosa-associated lymphoid tissue lymphoma. It is proven that HP colonization increases the risk of gastric cancer up to approximately 68 fold[4]. HP has also been designated a class I carcinogen by the WHO[5].

Significant differences in the prevalence of HP infection may be seen both worldwide as well as in various parts of any single country. HP prevalence is relatively high in developing countries. Among

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children, it varies widely, ranging from 10% to 80% depending on age and the factors mentioned above for the general population[2]. Epidemiological data from a study carried out in Poland in 2011 to 2012 showed that the prevalence of HP infection reaches 9.8% in healthy children and 14.4% in cystic fibrosis (CF) patients[6].

CF has an especially significant effect in the gastrointestinal tract; that is, along side that in the lungs. The decreased incidence of peptic ulcers in CF population was reported since the late 1990's[7,8]. It has been said about the so called "CF paradox" that patients with impaired duodenal bicarbonate secretion and unbuffered gastric acid have a lower inclination to ulceration then general population. Only recently has it been demonstrated that the CF patients have the entire spectrum of duodenal alterations, including duodenal ulcers[9].

Many investigators have studied the criteria for diagnosis of HP infection by different tests and methods, but the reliability of them for CF patients still remains controversial. Given that the incidence of HP infection and the spectrum of changes caused by this pathogen in CF patients is the same as in the general population, we aimed to assess the reliability of the two most popular noninvasive tests – urea breath test (UBT) and fecal test (FT) for diagnosis of HP infection in CF patients.

Patients and Methods

The study group consisted of seventy nine CF patients (38 females and 41 males) of Polish origin aged 3 to 39 years. The exclusion criteria comprised of:

Having taken oral or intravenous antibiotics (with the exception of azithromycin) for 3 months prior to the investigation;

Having taken proton pump inhibitors (PPI) for 3 months prior to the investigation.

The control group consisted of 49 healthy subjects of Polish origin aged 4 to19 years, who did not receive intravenous, oral antibiotics, or PPIs for four weeks prior to the investigation. The study was part of the project titled "PL0361/Good diagnosis - treatment-life" which was carried out by the First Specialist Clinical Hospital in Zabrze (Poland) for evaluating the incidence of gastrointestinal diseases in randomly selected children.

The genotypes of the studied CF patients were as follows: F508del/F508del (n=36), F508del/-(n=14), F508del/2143delT (n=3), F508del/CFTR del2,3(21kb) (n=2), F508del/2183AA>G (n=3), F508del/3849+10kbc>T (n=2), F508del/1717-1G-A (n=2), F508del/N1303K (n=1), F508del/3272-26A>G (n=1), F508del/3659delC (n=1), F508del/G1244E (n=1), F508del/G542X (n=1), F508del/R553X (n=1), G542X/- (n=2), N1303K/- (n=1), N1303K/3272-26A>G (n=1), R533X/- (n=1), 3849+10kbc>T/- (n=1), non detected -/- (n=5).

In all CF patients, the *Z-score* for body height and weight, fecal elastase-1 concentration, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), International Normalized Ratio (INR), and the *Pseudomonas aeruginosa* (PA) colonization were assessed. The FEV1 was determined in subjects older than 6 years. In patients with fecal elastase-1 concentrations higher than 100 μg/g, fecal fat excretion was determined to establish pancreatic sufficiency[10-12]. In addition, the socioeconomic status (e.g., residence in an urban or rural region) of the CF patients was assessed.

The presence of HP was evaluated in all subjects using the ^{13}C isotope-labeled UBT. The test was performed after a minimum of 6 hours of fasting. Two breath samples were collected; the first at baseline, and the second at 30 minutes after swallowing (drinking) a 200 ml of orange juice which contained 50 mg of ^{13}C-labeled urea. To collect the breath samples, 650 ml aluminized bags connected to one-way valves were used. Measurement of the $^{13}CO_2/^{12}CO_2$ ratio was carried out using an ^{13}C-infrared isotope analyzer system (IRIS, Wagner Analysen Technik, Bremen, Germany) with a cut-off value of delta over baseline (DOB) = 4‰[13].

Stool samples were collected from each subject. The samples were stored at –70°C until analysis took place. The presence of HP antigen in the stool was determined by an enzyme-linked immunosorbent assay using a commercially available monoclonal antibody kit (*H. pylori* Antigen ELISA Kit Diagnostic Automation, Calabasas, CA, USA). An aliquot of diluted stool sample was added to the wells, and the HP antigens, if was present, bind to the antibody. An enzyme conjugate was then added, which would then bind to the antibody-antigen complex. Excess enzyme conjugate was washed off, and the TMB Chromogenic substrate was added lastly. The enzyme conjugate catalytic reaction was stopped after 30 minutes. The results were read by a microwell reader compared in a parallel manner with calibrator and controls. Absorbance was measured at awavelength of 450 nm.

Table I. Positive *Helicobacter pylori* (HP) tests results detected by the urea breath test (UBT) and the fecal test (FT) in cystic fibrosis (CF) patients and healthy subjects.

Type of HP test	HP test positive (%) (n)		Statistical significance
	CF patients n = 79	Healthy subjects n = 49	
UBT	19.0 % (15)	16.3 % (8)	0.746
FT	15.2% (12)	14.3% (7)	0.892

Statistical Analysis

The comparison of the clinical parameters in patients with and without HP infection was performed using the Mann-Whitney test. The differences in distribution of the HP status between groups with different genotypes, with or without PA colonization, pancreatic sufficiency/insufficiency, and socioeconomic status ware analyzed using the χ^2 test. The influence of clinical parameters on the presence or absence of HP infection was determined with the use of logistic regression analysis. A value of $p < 0.05$ was considered asstatistically significant. All statistical analyses were performed with the use of Statistica 9.0 software.

Ethical Considerations

The protocol of the investigation was approved by the Ethical Committee of the Pozna University of Medical Sciences, Poland. Written informed consent was obtained from all subjects.

Results

The number of positive HP tests results detected by UBT and FT in CF patients and healthy subjects has been presented in Table I. In the group of 79 CF patients, HP infection was diagnosed in 15 (19.0%) based on UBT, and in 12 (15.2%) based on FT. Among 49 subjects of the control group, the infection was found in 8 (16.3%) based on UBT, and in 7 (14.3%) subjects based on FT. The prevalence of HP infection assessed with the use of both tests was comparable in both groups. However, the contrasting results between the two tests were found in 11.4% of cases in the CF group, and 6.1% of cases in the control group. Six CF patients were positive based on the UBT and negative on the FT, and three patients were negative on the UBT and positive based on the FT. Using the UBT as the standard, the sensitivity and specificity of the FT in CF patients were 60.0% (9/15) and 95.3% (61/64), respectively. Two of the healthy subjects were positive in the UBT and negative based on the FT, while one had a negative UBT result and a positive FT result. Using the UBT as the standard, the sensitivity and specificity of the fecal test in healthy subjects were 75.0% (6/8) and 97.5% (40/41), respectively (Table II).

The distribution of genotype, PA colonization, socioeconomic status, and pancreatic sufficiency was comparable in both the studied CF subgroups with concordant and divergent HP results (Tables III and IV).

In the analysis of logistic regression, no clinical factor was found to be an independent risk factor of HP infection in CF patients.

Table II. The results of *Helicobacter pylori* (HP) detection based on the urea breath test (UBT) and the fecal test (FT) in cystic fibrosis patients (CF) and healthy subjects (HS) .

Type of HP test		CF		HS	
		UBT			
		Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
FT	positive n (%)	9 (11.4%)	3 (3.8%) *	6 (12.3%)	1 (2.0%)*
	negative n (%)	6 (7.6%)*	61 (77.2%)	2 (4.1%)*	40 (81.6%)

*Discordant results between UBT and FT

Table III. *Pseudomonas aeruginosa* (PA) colonization, pancreatic sufficiency, and socioeconomic status in cystic fibrosis patients with concordant and divergent *Helicobacter pylori* (HP) test results based on the fecal test and the urea breath test.

Clinical parameters		HP test results		Statistical significance
		+/–	+/+ and –/–	
PA	Yes	5	36	n.s.
	No	4	34	
Pancreatic sufficiency	Yes	1	9	n.s.
	No	8	61	
Socioeconomic status	Urban	3	40	n.s.
	Rural	6	30	

+/– discordant HP test results in FT and UBT
+/+ concordant, positive HP test results in FT and UBT
–/– concordant , negative HP test result in FT and UBT

Discussion

All of the participants in our study, including the healthy subjects (HS) and the CF patients, were examined in order to detect HP infection using two different tests, UBT and FT, which are, according to many authors, comparable or even equivalent in terms of sensitivity and specificity[14,15]. Among the non-invasive HP diagnostic tests, UBT and FT have higher accuracy than serological or urinary antibody-based tests[16,17]. FT is currently recommended as it is a cheaper examination, is easier to perform than the UBT, and has also been shown to be successful in children[18,19].

The results of the present study have shown no clinical superiority of either of the two tests in regards to the HP detection in CF patients. As compared with the control group, CF patients demonstrated an increased number of conflicting results between UBT and FT (11.4% vs. 6.1% respectively); however, this difference has not reached statistical significance. In light of the presented facts, the doubt seems to be justified about whether the UBT and FT are really equally reliable in the evaluation of HP infection in CF patients. This is especially so, given that only 50% (9 out of 18) of them obtained concordant positive results in both tests which would confirm HP infection. The contrasting results between the two tests were found in 6.1% of cases in the HS group, and 11.4% of cases in the CF patients group. The percentage of discordant results in our study is considerably lower than reported by Masoero et al[20], and is comparable with the results obtained by Queiroz[21]. In Masoero's[20] studies, the divergent results between the FT and UBT were found in 30% of patients previously treated for HP infection, and in 19% of untreated patients. However, in their study, the main discrepancy was that there was a positive FT associated with a negative UBT. In our study, on the contrary, it was that there was a positive UBT associated with a negative FT (4.1% - HS, 7.6% - CF). Such a diagnostic divergence, as obtained by Masoero et al[20], and also reported by other authors[22-24], may be explained by the presence of degenerative or

Table IV. The prevalence of concordant and divergent *Helicobacter pylori* (HP) test results in cystic fibrosis (CF) patients with different genotypes.

CF mutations	HP test results		Statistical significance
	+/–	+/+ and –/–	
Severe/severe	4	46	n.s.
Others	4	22	

+/– discordant HP test results in FT and UBT
+/+ concordant, positive HP test results in FT and UBT
–/– concordant , negative HP test result in FT and UBT

coccoid HP forms of HP. Interestingly, it has recently been demonstrated that HP, beside the well-known spiral form, may exist in these two other stages: coccoid and degenerative. When exposed to detrimental environmental circumstances, the viable, culturable, and virulent spiral HP forms may convert into a viable but non-culturable state of a coccoid. The degenerative forms of HP are pyknotic, non-culturable, coccoid forms of an essentially dead cell. The coccoid and degenerative forms cannot be cultured but their gene material can be detected by PCR, and their antigens may be detected in the feces[25-27]. This can possibly explain the positive results of the FT obtained in the case of a negative UBT. Additionally, given that many CF patients are infected with PA, and that the solid-phase of HP antigen exhibits a cross reactivity with anti-Pseudomonas antibodies, it may be a possible explanation of the false positive FT results in CF patients colonized by this pathogen[28]. In this study, three CF patients had positive FT with negative UBT results; two of them were infected by PA. However, in our analysis, the vast majority of controversial results consisted of a positive UBT associated with a negative FT when considering CF patients. Six out of 15 the CF patients who were positive by the UBT, had negative FT results, and no plausible reason for why the FT failed to detect the pathogen in CF patients was found.

The sensitivity of the polyclonal FT for CF patients used in our study was 60.0%, while it was 75% for the control group. These results do not differ from those reported by other authors, which range within very wide limits, between 48.9% and 92%[29,30]. As reported Ritchie at al[31], differences in the antigenicity of the HP strains may affect the accuracy of FT in different populations. Therefore, sensitivity and specificity of FT should be tested in each population before its use in the management of HP infection. It is possible that the HP strains differ antigenically to a greater extent in CF patients who are undergoing chronic antibiotic therapy than of those strains in healthy subjects, and this may affect the variation in sensitivity of FT.

Conclusions

The small number of CF patients included in the analysis results from the relatively low prevalence of HP infection among CF patients (14.4%) in Poland[6]. Hence, the statistical power of the study results does not allow one to draw unequivocal clinical conclusions, the question of whether UBT and FT are equally reliable in the terms of HP detection in CF patients still remains actual and requires further investigation.

It is important to underline that the gold stadard among the non-invasive methods of HP detection remains UBT, and that since there is convincing evidence of false positive FT results in the CF patients mentioned above[28], we suggest that UBT is kept as the standard method for HP detection in CF patients, at least until obtaining the reliable and valid results allowing to change such an approach.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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