International Journal of Infectious Diseases (2005) **9**, 21—26

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INTERNATIONAL SOCIETY FOR INFECTIOUS DISEASES

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HpSA fecoprevalence in patients suspected to have *Helicobacter pylori* infection in Istanbul, Turkey

Ö. Büyükbaba-Boral[a,*], M. Küçüker-Anğ[a], G. Aktaş[a], H. İşsever[b], Ö. Anğ[a]

[a]*Departments of Microbiology and Clinical Microbiology, Istanbul Faculty of Medicine, Istanbul University, 34390 Çapa, Istanbul, Turkey*
[b]*Public Health, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey*

Received 2 October 2003; received in revised form 6 February 2004; accepted 28 April 2004

Corresponding Editor: Jonathan Cohen, Brighton, UK

KEYWORDS
Fecoprevalence;
Helicobacter pylori;
Turkey

Summary

Objective: This study aims to investigate the *Helicobacter pylori* antigen in the stool samples of patients suspected to have *H. pylori* infection. It also aims to determine the fecoprevalence in different age groups and to look for the relationship between the clinical symptoms seen in patients and the existence of *H. pylori*. Current information in Turkey is insufficient.
Methods: The *Helicobacter pylori* antigen was investigated in the stool samples of 445 patients of whom 148 were in the 2—15 year age group, 96 in the 16—30 year age group, 85 in the 31—40 year age group and 116 in the 41—48 year age group. The main clinical complaints of the patients (stomach pain, heart burn, indigestion, gas, nausea, vomiting, diarrhea, abdominal pain) were recorded.
Results: The *Helicobacter pylori* antigen was found in 36.6% (163/445) of the patients and in the statistical evaluation made for different age groups, a significant linear relationship was found between age and infection ($\chi^2 = 14.77$, $p = 0.002$).
Conclusion: It was found that stomach pain was seen at a higher rate in patients with *H. pylori* antigen compared to those without it. The difference was highly statistically significant ($\chi^2 = 117.70$, $p < 0.001$, OR = 20.36, 95% CI = 10.56—39.27).

* Corresponding author. Tel.: +90 212 635 11 86; fax: +90 212 635 11 86.
E-mail address: obboral@yahoo.com (Ö. Büyükbaba-Boral).

1201-9712/$30.00
doi:10.1016/j.ijid.2004.04.014

Introduction

Helicobacter pylori causes several gastric diseases ranging from gastritis to peptic ulcer. Furthermore, *H. pylori* is considered to play a role in the development of gastric cancer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Infection with *H. pylori* is very common throughout the world. Although there are significant differences in the prevalence of *H. pylori* infection, both within and between countries, it occurs in approximately 40—50% of the population in developed countries and 80—90% of the population in developing countries.[1—3]

Various diagnostic tests are available for the detection of *H. pylori* infection and these can be grouped into invasive and non-invasive. Tests that require endoscopy include the biopsy urease test, histology, culture, PCR-based methods, and phase-contrast microscopy of gastric tissue. Diagnostic tests that do not require endoscopy include the ^{13}C-or ^{14}C-labeled urea breath test, serology, the string test and the stool antigen enzyme immunoassay (EIA).[4]

Over the last few years, although *H. pylori* has been cultured from stool samples, it has been shown that viable organisms are present only in a small percentage of cases.[4—7]

Despite the difficulties encountered in the culture from stool samples, the fact that dead and/or alive organisms were present in all the stools raised the possibility of developing a new non-invasive diagnostic test for the detection of the *H. pylori* antigen in stools. Premier Platinum HpSA (Meridian Diagnostic Inc., Cincinnati, Ohio, USA) has developed an in-vitro qualitative enzyme immunoassay commercial kit which states that it can detect *H. pylori* protein antigens when present at a concentration of $\geq$184 ng/ml of feces.[4]

Because information on the prevalence of *H. pylori* infection is insufficient in Turkey, this study aims to investigate the *H. pylori* antigen in the stool samples of patients suspected to have *H. pylori* infection and to conduct a statistical evaluation with respect to age groups. This will then determine the fecoprevalence in different age groups and look for the relationship between the existence of the *H. pylori* antigen and the clinical symptoms.

Materials and methods

Patients

This study investigated outpatients who presented at the Department of Gastroenterology of the Internal Medicine Clinic and the Pediatric Gastroenterology Clinic of the Istanbul Faculty of Medicine who were suspected to have *H. pylori* infection and whose stool samples were sent to the Department of Microbiology and Clinical Microbiology for *H. pylori* antigen examination. The *H. pylori* antigen was investigated in the stool samples of 445 patients suspected to have *H. pylori* infection, of which 148 were in the 2—15 year age group, 96 in the 16—30 year age group, 85 in the 31—40 year age group and 116 in the 41—85 year age group. Data relating to these patients were recorded using a questionnaire. The patients were asked their age and main clinical complaints, such as stomach pain, heart burn, indigestion, gas, nausea, vomiting, diarrhea, and abdominal pain. Patients who complained of discrete stomach pain or gastric pain were placed in the stomach pain group, while those who complained of pain more diffuse over the abdomen were placed in the abdominal pain group.

Antigen detection in stool

A commercial kit was used: Premier Platinum HpSA (Meridian Diagnostic, Ohio, USA). This sandwich enzyme immunoassay procedure employs polyclonal anti-*H. pylori* antibodies. The stool samples were stored at 2—8 °C for up to three days (for >3 days at −20 °C) before the test. A small portion of the specimen in diluent, positive and negative controls were added to each antibody-coated microwell. After adding one drop of enzyme conjugate to each well, the plate was incubated for one hour at room temperature, then the wells were washed five times to remove the unbound material. Two drops of substrate were added and incubated for 10 minutes at room temperature. In the presence of bound *H. pylori* antigens, a color develops. One-drop stop solution was added and the results were evaluated by spectrophotometric determination of absorbance at dual wavelength (450/630 nm). A Δ 450/630 of 150 was considered positive.

Statistical analysis

Statistical analysis was performed with the χ^2 test, Fisher's Exact test, and step-wise logistic regression analysis.

Results

The *H. pylori* antigen was detected in 163 (36.6%) patients. The distribution of the patients with *H. pylori* infection with respect to age group is given in Table 1. In the statistical analysis carried out with

Table 1 The age group distribution of the patients whose stool samples were positive for the *Helicobacter pylori* antigen.

Age group	HpSA (+)	
	n	(%)
2—15 (*n* = 148)	37	25.0
16—30 (*n* = 96)	36	37.5
31—40 (*n* = 85)	36	42.3
41—78 (*n* = 116)	54	46.5

$\chi^2 = 14.77$, $p = 0.002$.

respect to age groups, a significant linear relationship between age and infection ($\chi^2 = 14.77$, $p = 0.002$) was observed. The clinical symptoms of the patients and the positive and negative results obtained from the stool samples are compared in Table 2.

Among the 163 patients in whom the *H. pylori* antigen was found, 152 (93.2%) had stomach pain as the most common complaint, while among the 282 patients in whom *H. pylori* antigen was not found, 114 (40.4%) had stomach pain as the common complaint. The difference was statistically significant ($\chi^2 = 117.70$, $p < 0.001$, OR = 20.36, 95% CI = 10.56—39.27). When complaints of the patients with the *H. pylori* antigen (stomach pain, stomach pain + nausea and vomiting, stomach pain + indigestion + gas) were compared with the complaints of the patients without the *H. pylori* antigen, it was found that the difference was highly significant (Table 2).

A logistic model was constructed in which the HpSA (+)/(—) variable was taken as the dependent variable, and stomach pain, stomach pain + indigestion + gas, stomach pain + nausea and vomiting, which were found to be statistically significant in the bivariate analysis, were taken as independent variables and the model was solved by a stepwise logistic regression analysis. The results of the analysis are shown in Table 3. Variables showing HpSA positivity were stomach pain, stomach pain + indigestion + gas. In HpSA (+) cases, the presence of stomach pain was 22.51 times higher than in HpSA (—) cases (OR = 22.51, 95% CI 11.47—44.19), and the presence of stomach pain + indigestion + gas was 5.16 times (OR = 5.16, 95% CI 1.04—25.48) higher than in HpSA (—) cases. There was a significant relationship between the absence of the *H. pylori* antigen and the symptoms of stomach pain + heart burn, nausea and vomiting, abdominal pain and diarrhea.

Discussion

Helicobacter pylori is the cause of gastritis, gastric and duodenal ulcer. In addition, the association between *H. pylori* and gastric carcinoma and lymphoma is becoming clear.[8] Moreover, *H. pylori* is also being investigated for contributing to extragastrointestinal disorders, such as impaired growth, coronary heart disease, diabetes and gallstone disease.[9]

Currently available tests for the diagnosis of *H. pylori* infection have relatively high sensitivities and specificities but each has its limitations in clinical application. Urease-based biopsy tests require endoscopy and are not reliable in patients taking proton-pump inhibitors. Histologic examination also requires endoscopy and is subject to sampling error. Its accuracy is dependent on the stain selected and on the pathologist's skill. Serology is inexpensive but is not reliable for determining the presence of active infection. On the other hand, it has been found that serology in the post-treatment follow-up period has 97% sensitivity and 95% specificity. It has also been reported that a 50% decrease in the antibody titer six months after the treatment indicates the success of the treatment.[4,10—13]

Overall, studies using pretreatment stool *H. pylori* antigen tests have shown that the sensitivity and specificity of stool antigen testing are comparable to histology, culture or urea breath test (UBT).[14—26]

Sykora et al.[22] investigated the biopsy samples of 91 children (average age 12.6 ± 3.5) with a

Table 2 Comparison of clinical symptoms between HpSA positive and negative patients.

Clinical symptoms	HpSA (+)		HpSA (—)		*p* two-tailed significance	OR	95% CI
	n = 163	(%)	*n* = 282	(%)			
Stomach pain	55	33.7	50	17.7	$p = 0.0002$	2.36	1.51—3.69
Stomach pain + heart burn	25	15.3	29	10.3	$p = 0.15$	1.58	0.89—2.80
Stomach pain + nausea + vomiting	35	21.5	0		$p < 0.001$	156,09	9.49—2566
Stomach pain + indigestion + gas	37	22.7	35	12.4	$p = 0.006$	2.07	1.24—3.45
Nausea + vomiting	3	1.8	68	24.1	$p < 0.001$	16.94	5.3—54.85
Abdominal pain	7	4.3	57	20.2	$p < 0.001$	5.64	2.50—12.70
Diarrhea	0	—	43	15.2	$p < 0.001$	59.39	13.62—972.27

OR = odds ratio; CI: confidence interval; *a gallstone in one patient.

Table 3 Results of logistic regression analysis.

Independent variables	B	SE	df	Significance	OR	95% CI
Stomach pain	3.11	0.34	1	$p < 0.001$	22.51	11.47—44.19
Stomach pain + indigestion + gas	1.64	0.81	1	$p = 0.044$	5.16	1.04—25.48
Constant	−12.03	15.03	1	$p = 0.423$		

OR = odds ratio; SE: standard error; df: degrees of freedom.

rapid urease test and histology and found *H. pylori* infection in 31 (34.1%) of cases. They observed *H. pylori* antigen in the stool samples of 28 of these children and reported the sensitivity of the test as 90.3%. They did not find *H. pylori* antigen in the stool samples of the 60 children who were found to be negative in the histologic examination and rapid urease test, thus specificity was 100%. Van Doorn et al.[24] searched for *H. pylori* infection in 106 children with an average age of 8.5 years by using culture and histologic methods and compared their findings with EIA for *H. pylori* antigen in the stool. They found *H. pylori* infection in 30 (28%) children by culture and histologic investigation, and observed the *H. pylori* antigen in stool samples of all of these children. They reported the sensitivity of HpSA (EIA) as 100% and specificity as 92%. Vaira et al.[20] observed that in one prospective multicenter trial with 501 patients with *H. pylori* infection, the stool antigen for *H. pylori* was positive in 256 of 272 patients (sensitivity, 94.1%; 95% CI, 91—97%). This was determined by histology and a rapid urease test or culture. Of the 219 patients without infection, 201 tested negative to HpSA (EIA) (specificity, 91.8%; 95% CI, 87—95%). Gisbert and Pajares[27] evaluated the results from 43 studies (4769 patients). They investigated the validity of the HpSA (EIA) test in the diagnosis of *H. pylori* infection in patients who were not under treatment. Their results show that sensitivity is 92.4% (95% CI = 91—93%), specificity is 91.9% (95% CI = 91—92%), PPV is 92.1% (95% CI = 91—93%), NPV is 90.5% (95% CI=90—91%) and that HpSA (EIA) test in stool is a good non-invasive method for *H. pylori* diagnosis.

In Turkey there have been previous studies where *H. pylori* fecoprevalence has been investigated. In one previous study carried out in Turkey, *H. pylori* infection was diagnosed in 55% of 22 children with repeating abdominal pains whose average age was 12.2 ± 2.0 (median 12.5, range 9—15) by using a rapid urease test and histologic investigation.[28] In the present study, *H. pylori* infection was detected in 25% of children in the 2—15 year age group by using HpSA (EIA) and no relationship was found between abdominal pain and *H. pylori* infection.

In a study in Turkish children in the 1—4 year age group who lived in Germany and who were thought to be in the high risk group, *H. pylori* prevalence was found to be 27%. A 35% decrease in infection was observed after one year in the same group of children and *H. pylori* colonization was not found to be persistent at young ages. Furthermore the investigators mentioned that the use of penicillin and macrolides had an important role in the decrease of infection.[25]

Sykora et al.[22] determined *H. pylori* infection in 34.1% of 91 children with symptoms of indigestion at the average age of 12.6 ± 3.5 years by using the rapid urease test and histologic investigation, in Slovakia. Cheng et al.[29] studied *H. pylori* infection prevalence in Taiwan among children and young adults to determine differences between urban and rural settlement areas. They divided 567 children and young adults into six groups (0—3, 3—6, 6—9, 9—12, 12—15, and 15—18 years) and directed a questionnaire to at least 30 persons from each group. They found that the total prevalence was 13.7%, that the minimum prevalence (2%) was observed in the 0—3 year age group and that the prevalence increased linearly with age, reaching a maximum level of 23.8% in the 9—12 year age group before declining. Furthermore, they found that the settlement area, sex, education of the mother, jobs of the parents and whether or not there was a peptic ulcer case in the family did not influence *H. pylori* infection. They found that stomach pain ($p = 0.001$) and education of the father ($p = 0.039$) had an important relationship with *H. pylori* infection. Similarly, this present study showed that stomach pain had a significant correlation with the *H. pylori* infection ($p = 0.0002$).

In a fecoprevalence study carried out in Canada among Indian and Eskimo children between six months and 12 years of age, in which *H. pylori* antigen was investigated in stool samples by using EIA, prevalence was reported as 56% (163/92). Sex, owning pets, serum hemoglobin and the existence of occult blood in stool had no relation with the *H. pylori* prevalence. However, researchers have pointed out that the use of toilets outside the house was influential in *H. pylori* infections occurring at an early age such as six months.[30] In the present study,

despite the suspicion of *H. pylori* infection, the prevalence in the 2—15 year age group was 25%, lower than prevalence found in the above study. This may be due to the fact that the present study was carried out on children living in areas benefiting from a good infrastructure in Istanbul.

Malfertheiner et al.,[31] in the Maastricht 2-2000 Consensus Report, underlined the necessity of *H. pylori* diagnosis and treatment in adults under 45 years of age with persistent dyspepsia (the age level may change locally). The results of the present study show that the probability of *H. pylori* infection is high in patients with complaints of stomach pain, nausea, vomiting, indigestion and gas. It is necessary to test these patients for *H. pylori*.

This study is the first prevalence search based on the determination of the *H. pylori* antigen in stool samples in Turkey. The results have shown that the prevalence of *H. pylori* infection in Turkey is as high as 36.6%.

Conflict of interests: No conflict of interest to declare.

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