The clinical importance of *Helicobacter pylori* antigens detected in the dental plaque and feces

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ABSTRACT

Purpose: It is expected that *H. pylori* residing outside the stomach influences the results of the stool test. The aim of the study was to investigate the occurrence of *H. pylori* antigens in dental plaque and feces of the patients with *H. pylori* infected and non-infected stomachs.

Materials and methods: The study was conducted in 188 dentate patients, 107 with *H. pylori* infected and 81 non-infected stomachs. Stomach infection with *H. pylori* was evaluated with CLO test, histology and culture. The stomach was classified as infected if at least two of three tests (CLO test, culture, histology) were positive and as non-infected if all three tests were negative. Dental plaque was taken only from the natural teeth. On the day of the gastroscopic examination or on the next two days a stool sample was collected for *H. pylori* antigens testing. *H. pylori* antigens in dental plaque and feces were determined by immunological method.

Results: In 60.8% of subjects with an infected stomach, *H. pylori* antigens were present both in the dental plaque and feces, in 37.4% in feces only, in 0.9% only in the dental plaque, and in 0.9% neither in the dental plaque nor feces. In 46.9% of subjects with a non-infected stomach, *H. pylori* antigens were found neither in the dental plaque nor feces, in 24.7% both in the dental plaque and feces, in 23.5% only in the dental plaque, and in 4,9% only in feces.

Conclusions: There is a weak association between the occurrence of *H. pylori* antigens in feces and the dental plaque, and also between the occurrence of the antigens in the dental plaque and stomach infection.

Key words: *Helicobacter pylori* antigens, immunoassay, dental plaque, feces

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INTRODUCTION

In the last decade, a test assessing the occurrence of *Helicobacter* (*H.) pylori* antigens in feces has been applied and popularized [1,2].

At present, it is used in the evaluation of stomach infection before and after eradication therapy both in adults and children [2,3].

Unfortunately, some number of positive test results (approximately 10%) in subjects with a non-infected stomach imply that extragastric bacteria influence the test result [4-6].

By using the *H. pylori* antigen test it was shown that *H. pylori* occurs not only in feces but also in dental plaque and saliva [7-10].

Therefore, it cannot be excluded that detection of *H. pylori* antigens in feces is a consequence of the presence of *H. pylori* bacteria only in some extragastric locations, e.g. in the oral cavity. This is theoretically possible as *H. pylori* may pass through the stomach without causing an infection [11].

The aim of this study was to test the hypothesis that there is association in the occurrence of *H. pylori* antigens in feces and dental plaque in subjects both with infected and non-infected stomachs.

MATERIALS AND METHODS

Study subjects

One hundred and eighty eight patients between the ages of 19-77 years were enrolled in the study; 107 with *H. pylori* infected and 81 non-infected stomachs (Table 1).

Table 1. Patients' characteristics

Age (years; median, range)	54.0 (19-77)
Gender (M/F)	73/115
Smokers (%)	40 (21.3)
Alcohol usage (%)	41 (21.8)
Diagnosis	
Gastritis (%)	166 (88.3)
Peptic ulcer disease (%)	22 (11.7)

They had natural or a combination of natural and artificial teeth.

The inclusion criteria were as follows: good general condition (an inter-view and medical examination), no chronic or devastating diseases, no antibiotics taken within the last month, and no *H. pylori* eradication treatment in the past.

Samples collection and processing

Each patient had a gastroscopic examination with biopsies of gastric mucosa taken from the prepyloric and the gastric body areas, one for a urease test, one for culture, and two for histological examination. The *H. pylori* infection in the stomach was determined by an urease test (Campylobacter-Like Organism - CLO test), pre-pared in the Physiology Department of the Medical University in Białystok, using methods described by Marshall et al. [12].

The results of test were considered to be positive if a color change from orange to pink was observed within 24 hours. The sensitivity and specificity of CLO test in relation to the histological examination, culture, and stool test were 84.3% and 88.4%, 87.5% and 83.5%, and 75.4% and 87.5 %, respectively [13].

Specimens for culture were collected into transport medium (Por-tagerm-pylori, bioMerieux, France) and following homogenization were inoculated on selective Pylori-Agar (bioMerieux, France) and nonselective Columbia agar enriched with 5% sheep blood (Oxoid, UK). The culture was conducted under microaerophilic conditions for 7 days at 37^{o}C. Specimens for histological examination were placed in buffered formalin and then processed and stained with hematoxylin-eosin and Giemsa. The microscopic assessment of the preparations was performed by two experienced histopathologists who did not know the results of the other tests. Gastritis was assessed on a 4-step scale (0-3) including neutrophil (activity) and mononuclear cell infiltration (inflammation) and *H. pylori* density [14].

The stomach was classified as infected if at least two of three tests (CLO test, culture, histology) were positive and as non-infected if all three tests were negative (Table 2).

Table 2. Qualification of stomach infection with *H. pylori* on a base of three tests

CLO test	**histology**	**culture**	**n(%)**
+	+	+	90(84.1)
+	-	+	5(4.7)
-	+	+	4(3.7)
+	+	-	8(7.5)

Dental plaque was collected only from the natural teeth, at least 2 mg from each subject, always in the morning before breakfast, oral hygienic practices, and gastroscopic examination. The plaque examination was started soon after collection. On the day of the gastroscopic examination or on the next two days after it a stool sample was collected for *H. pylori* antigens testing.

Helicobacter pylori antigen test

The determination of *H. pylori* antigens in dental plaque and feces was conducted in accordance with the manufacturer's instruction (Amplified IDEIA™, Hp StAR ™, Oxoid, UK).

In brief, the sample and horseradish peroxidase labeled monoclonal antibodies were added in one step to the monoclonal antibody- coated microwells of the microtitration plate, using a sandwich technique.

After incubation, the microwells were washed with phosphate buffer to remove the unbound antibody conjugates and tetramethyl-benzidine was added.

Bound horseradish peroxidase oxidized tetramethylbenzidine to a blue colored product.

The reaction was stopped with sulphuric acid which changed the color from blue to yellow.

The intensity of the color was measured spectrophotometrically.

Modification of the method used for determination of *H. pylori* antigens in dental plaque relied on preliminary incubation of the plaque for 72 hours in microaerophilic conditions [7].

Statistical Analysis

The results were analyzed using Mann-Whitney U test (Statistica 8.0). The differences were considered to be statistically significant at $p<0.05$. The sensitivity and specificity of *H. pylori* antigen stool test in relation to the occurrence of stomach infection were calculated according to standard methods.

RESULTS

In 60.8% of subjects with an infected stomach (positive results in at least two of three gastric tests), *H. pylori* antigens occurred both in the dental plaque and feces, in 37.4% only in feces, in 0.9% only in the dental plaque, and in 0.9% the antigens of *H. pylori* were present in neither the dental plaque nor feces (Table 3).

In 46.9% of subjects with a non-infected stomach no presence of *H. pylori* antigens was found in either the dental plaque or feces, in 24.7% antigens occurred in both the dental plaque and feces, in 23.5% only in the dental plaque, and in 4.9% only in feces (Table 3).

In 10.6% of all subjects, *H. pylori* antigens were found in the dental plaque and feces but no stomach infection was found (Table 4).

In these two groups, the histology of gastric mucosa characteristic for *H. pylori* infection did not occur (Table 5).

The sensitivity and specificity of the test for the presence of *H. pylori* antigens in feces in relation to the occurrence of stomach infection for the entire population studied amounted to 98.5% and 71.1%, respectively.

If excluding those for whom positive results of the stool test were not associated with stomach infection but were associated with the presence of *H. pylori* antigens in the oral cavity, the specificity of the stool test increases to 93.5%.

Table 3. The distribution of *H. pylori* antigens in the dental plaque and feces of subjects with infected and non-infected stomachs

	***H. pylori* antigens**		
	plaque	**stool**	**n(%)**
Infected stomach (n=107)	+	+	65(60.8)
	+	-	1(0.9)
	-	+	40(37.4)
	-	-	1(0.9)
Non- infected stomach (n=81)	+	+	20(24.7)
	+	-	19(23.5)
	-	+	4(4.9)
	-	-	38(46.9)

In 10.2% of all subjects, the presence of *H. pylori* antigens was documented in the plaque but no stomach infection and *H. pylori* antigens in feces were found.

Table 4. *H. pylori* status of gastric mucosa in relation to *H. pylori* antigens in the dental plaque and feces

plaque *H. pylori* antigens	**stomach *H. pylori* infection**	**stool *H. pylori* antigens**	**n(%)**
+	+	+	65(34.6)
+	-	+	20(10.6)
+	+	-	1(0.5)
+	-	-	19(10.2)
-	+	+	40(21.3)
-	-	+	4(2.1)
-	-	-	38(20.2)
-	+	-	1(0.5)

Table 5. Histological characteristics of the gastric mucosa in relation to the presence of *H. pylori* antigens in the dental plaque and feces (median, range)

	antrum		**corpus**	
***H. pylori* antigens**	**Inflammation**	**activity**	**Inflammation**	**activity**
infected stomach (total)	3(1-3)	2(0-3)	1(0-3)	2(0-3)
plaque (+) stool (+)	3(1-3)	2(0-3)	1.5(0-3)	2(0-3)
plaque (+) stool (-)	3	3	1	2
plaque (-) stool (+)	3(1-3)	3(1-3)	2(0-3)	2(0-3)
plaque (-) stool (-)	3	2	1	2
non-infected stomach (total)	1(0-3)*	0(0-2)*	0(0-3)*	0(0-2)*
plaque (+) stool (+)	1(0-3)*	0(0-2)*	0(0-2)*	0(0-2)*
plaque (+) stool (-)	0(0-2)	0(0-2)	0(0-2)	0(0-2)
plaque (-) stool (+)	1(0-1)*	1(0-1)*	0(0)*	0(0)*
plaque (-) stool (-)	1(0-2)	1(0-2)	0(0-3)	0(0-2)

$p < 0.001$ vs infected stomach

DISCUSSION

The results of this study have shown that there is a weak association between the occurrence of *H. pylori* antigens in feces and the dental plaque, and also between the occurrence of antigens in the dental plaque and stomach infection. Full correspondence of results determining *H. pylori* antigens in dental plaque and feces with stomach infection was found only in 54.8% of subjects. Assuming that dental plaque is a basic location of *H. pylori* in the oral cavity [15,16], infection of the stomach with this bacterium (positive results in at least two of three gastric tests) combined with the simultaneous presence of their antigens in feces and absence in dental plaque implies that in a number of subjects with infected stomachs the oral cavity remains uninfected. It constitutes indirect evidence that stomach infection may occur without a corresponding infection of the oral cavity. In 0.9% of subjects, the stomach is infected even with the absence of *H. pylori* antigens in the oral cavity and feces. In 0.9% of subjects, the stomach is infected and *H. pylori* antigens are present in the oral cavity but not in feces. In both cases, an error in the assessment of *H. pylori* infection in the dental plaque, stomach or feces is likely. However, it should be noticed that the percentage of clearly erroneous results is small.

An interesting issue in subjects with a non-infected stomach is the occurrence of *H. pylori* antigens in dental plaque and their absence in feces or the presence of *H. pylori* antigens both in feces and in dental plaque. Since only a sufficiently large number of bacteria reaching the stomach, under favorable conditions, can cause its infection [11], it may be supposed that either the population of *H. pylori* in the oral cavity in these subjects was too small [17] or the bacteria were in a viable but non-culturable form [18,19].

In 24.7% of subjects with non-infected stomachs, the concomitant presence of *H. pylori* antigens in the dental plaque and feces was found. Apart from negative results of the three tests assessing the presence of bacteria in endoscopic specimens, also the inflammatory response of the gastric mucosa typical for *H. pylori* infection was not observed. Only advanced gastritis with no infection of *H. pylori* would allow us to suspect that an error in the microscopic examination of gastric mucosal specimens took place [20,21]. The simultaneous presence of *H. pylori* antigens in the dental plaque and feces without a stomach infection (10.6% of all subjects studied) might indicate that a positive stool test is related, in a number of cases, to the presence of *H. pylori* only in the oral cavity. In subjects with positive stool test but with a non-infected stomach and negative for plaque antigens, the extra-stomach population of *H. pylori*, e.g., oral bacteria from other locations than dental plaque, might be a source of *H. pylori* antigens in feces [9,10,19]. One may think therefore that proper oral hygiene might, in some extent, protect against *H. pylori* presence in the oral cavity, but no evidence for this was found in earlier studies [8,16,22].

The current results have shown that in subjects with positive stool test the stomach is infected only in 81.4%. On the other hand, 23.5% of subjects who have *H. pylori* antigens both in feces and in the dental plaque have a non-infected stomach. Based on positive results in two tests for the presence of *H. pylori* antigens (feces, dental plaque) it is not possible to confirm a stomach infection in an accurate manner, unless additional tests documenting a direct stomach infection are performed. Negative results of the tests documenting *H. pylori* infection in gastric mucosal specimens would indicate an extragastric source of *H. pylori* antigens in feces. Thus, the assessment of the presence of stomach infection exclusively on the basis of tests illustrating the presence of *H. pylori* antigens in feces or in dental plaque and feces possess a high risk of error, at least in a population with a high index of *H. pylori* infection [23,24].

CONCLUSIONS

Tests assessing the presence of *H. pylori* antigens in feces and dental plaque are helpful in diagnosing stomach infection, however, if relying on only these tests, a number of patients will require additional complementary tests due to the high percentage of false positive results.

Conflicts of interest

None declared.

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