The influence of *Helicobacter pylori* eradication therapy on the presence of *H. pylori* antigens in dental plaque and saliva

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ABSTRACT

Purpose: The aim of this study was to evaluate the presence of *H. pylori* antigens in the oral cavity (dental plaque and saliva) of patients undergoing systemic eradication therapy.

Materials and methods: The study was conducted in 49 subjects with *H. pylori* stomach infection. *H. pylori* antigens in dental plaque and saliva were evaluated with immunological method.

Results: In subjects with initial *H. pylori* oral infection, the presence of *H. pylori* antigens in the oral cavity 6 weeks after successful or unsuccessful *H. pylori* eradication therapy in the stomach was 47.0% and 50.0%, respectively. In subjects without initial oral infection with *H. pylori*, the presence of *H. pylori* antigens in the oral cavity 6 weeks after successful and unsuccessful eradication therapy in the stomach was 30.0% and 20.0%, respectively.

Conclusions: The immunological method detecting *H. pylori* antigens in the dental plaque and saliva cannot be recommended to evaluate the efficacy of *H. pylori* eradication in the oral cavity.

Key words: *Helicobacter pylori* treatment, immunoassay, gastric mucosa, oral cavity

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INTRODUCTION

Unsuccessful eradication of *H. pylori* results not only from increasing resistance of the bacteria against antibiotics, but also from the lack of an efficient method of bacterial elimination from extragastric locations [1-3]. The oral cavity is one of the sites where *H. pylori* may survive eradication therapy [1-3]. However, so far only a few studies have evaluated the presence of *H. pylori* in the oral cavity following eradication therapy [1,2,4,5].

Studies on *H. pylori* oral infection are much more difficult than those concerning the stomach, mainly due to the much more abundant bacterial flora in the oral cavity. There are over a dozen different bacterial strains in the stomach, with *H. pylori* being dominant, while the bacterial flora of the oral cavity includes several hundred different strains, and the *H. pylori* population is relatively small [6]. Furthermore, most methods used for the detection of *H. pylori* in the stomach cannot be applied to the oral cavity [7]. In recent years, a test evaluating the presence of *H. pylori* antigens with immunoassay has been used for the assessment of *H. pylori* oral and stomach infection [8-11]. Earlier it had been successfully applied for the determination of *H. pylori* antigens in stool and recommended for the evaluation of efficacy of *H. pylori* eradication therapy in the stomach [12].

The aim of this study was to evaluate the incidence of *H. pylori* antigens in dental plaque and saliva of patients undergoing a systemic eradication treatment.

MATERIALS AND METHODS

Study subjects

Forty-nine patients, men and women aged 24-72 years, were included in the study (Table 1). The inclusion criteria were good general health, normal basic laboratory tests, and no history of antibiotic therapy or other medication influencing the presence of *H. pylori* in the oral cavity at least one month preceding study entry.

Table 1. Characteristics of the 49 patients qualified for eradication therapy

Age (median, range)	51 (24-72)
Gender (M/F)	27 / 22
Smokers	17 (34.7%)
Alcohol users	14 (28.6%)
Diagnosis	
dyspepsia	18 (36.7%)
duodenal ulcer disease	26 (53.1%)
gastric ulcer disease	5 (10.2%)
H. pylori eradication (first / consecutive)	39 / 10
Number of natural teeth (mean ± SD)	17.2 ± 8.6
Denture users	27 (55.1%)
fixed	8 (16.3%)
removable	15 (30.6%)
fixed + removable	4 (8.2%)

Samples collection and processing

Each patient had a gastroscopy twice, i.e. prior to and 6 weeks after the completion of eradication therapy. During the gastroscopy, biopsy specimens of the gastric mucosa from the prepyloric and body regions were taken: one for rapid urease test (*Campylobacter*- like organism test - CLO test), two for histological examination and one for bacterial culture from each site. The CLO test was prepared in the Physiology Department of the Medical University of Białystok according to the method described by Marshall et al. [13]. The sensitivity and specificity of the 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 [8].

The biopsy specimens for the histological examination were placed in buffered formalin, processed using a standard method and assessed by experienced pathologists. The specimens taken for the bacterial culture were placed into transport medium (Portagerm pylori, bioMerieux), delivered immediately to the microbiology laboratory, inoculated into culture medium and cultivated in microaerophilic conditions for at least 7 days. The subject was considered infected if the results of at least two of the three tests used for detection of infection in endoscopically taken slices of the gastric mucosa (CLO test, histology, culture) were positive (Table 2).

Table 2. Gastric and oral cavity *H. pylori* infections in the population qualified for eradication therapy (n=49)

Saliva	Dental plaque	Gastric mucosa specimens			
H. pylori antigens		Culture	CLO-test	Histology	n
-	-	+	+	+	15
-	+	+	+	+	9*
+	+	+	+	+	9*
+	-	+	+	+	6
-	+	+	+	-	2
+	+	+	+	-	1
+	-	+	-	+	1
-	-	-	+	+	1
-	-	+	-	+	1
-	A	-	+	+	1
-	A	+	+	+	1
-	B	+	+	-	1
+	B	-	+	+	1

A – too small mass of dental plaque to perform examination; B – toothlessness; *- one subject did not complete the eradication therapy

Helicobacter pylori antigen test

The saliva and dental plaque were collected before each gastroscopic examination; i.e. prior to the initial gastroscopy and 6 weeks after the completion of eradication treatment. Each patient was asked to abstain from any oral hygiene procedures on the day of the study. The freshly collected plaque was placed into 0.15 mol/L NaCl solution and initial incubation in microaerophilic conditions for a 72-hour period was performed [9]; the aim of this procedure was to increase the number of bacteria in the studied samples. Each subject provided 5 mL of unstimulated saliva, which was subsequently centrifuged. The saliva sediment was then used to determine the presence of *H. pylori* antigens [10]. The oral cavity of the examined subject was defined as infected if the test for the presence of *H. pylori* antigens in dental plaque or saliva was positive. The detection of *H. pylori* antigens in dental plaque and saliva was performed according to the manufacturer's instructions provided with each kit, which was originally designed for detecting *H. pylori* antigens in stool. In brief, the prepared supernatant of the dental plaque or saliva was transferred to a well plate coated by the manufacturer with monoclonal antibodies against *H. pylori*. The solution containing monoclonal antibodies against *H. pylori* conjugated with horseradish peroxidase was also added to the wells.

After a 60-minute incubation, unbound anti-bodies were washed away and tetramethylbenzidine, a substrate for horseradish peroxidase, was added. The reaction was stopped by the addition of sulphuric acid. The yellow colour intensity was measured spectrophotometrically at 450 nm.

Antibacterial therapy

Eradication therapy was performed in 49 patients with stomach infection (a positive result in at least two of three tests used for evaluation of *H. pylori* infection in endoscopically taken samples) using a set of drugs: (1) Controloc (pantoprazole) 40 mg b.i.d., (2) Duomox (amoxicillin) 1000 mg b.i.d., and (3) Klacid (clarithromycin) 500 mg b.i.d. The therapy was continued for 7 days. The drugs were taken half an hour before meals with a half glass of water. The therapy was accepted as performed according to the study protocol if the patient had taken all medications designed for the therapy. During treatment and the subsequent 6 weeks, the patients did not change or modify oral hygiene procedures. Eradication therapy in the stomach was considered successful if the result of none of the three tests evaluating the presence of *H. pylori* in the endoscopically taken specimens 6 weeks after the treatment was positive. The eradication of *H. pylori* in the oral cavity was defined successful, if 6 weeks after the treatment, no *H. pylori* antigens were found in the dental plaque or saliva.

Statistical analysis

The results were analysed statistically using Fisher's exact test (Statistica 8.0). Statistical significance was accepted at $p<0.05$.

RESULTS

Of the 49 subjects with *H. pylori* stomach infection (a positive result in at least 2 of 3 tests) 2 subjects did not take all medications and were excluded from final analysis. In the remaining 47

subjects with an infected stomach who completed the eradication therapy according to the study protocol, 27(57.4%) had at baseline the presence of *H. pylori* antigens in the oral cavity (dental plaque or saliva) (Table 2). Among the 27 subjects with *H. pylori* oral infection, *H. pylori* antigens were found in the saliva of 8 subjects, in the dental plaque of 10 subjects, and in both the dental plaque and saliva of 9 subjects (Table 3). The therapy led to *H. pylori* eradication in the stomach and oral cavity in 55.3% (26/47) and 55.6% (15/27) of subjects, respectively, but only in 29.6% (8/27) of subjects in both.

Table 3. Presence of *H. pylori* antigens in the oral cavity before and after eradication therapy; patients with no antigens in the oral cavity prior to eradication therapy but with such antigens after treatment are also included

	Before treatment	Post treatment	
		Eradication successful in the stomach	Eradication unsuccessful in the stomach
Dental plaque	10	7 **	3*
Saliva	8	3*	2
Dental plaque and saliva	9	0	3*
Total	27 / 47 (57.4%)	10 / 27 (37.0%)	8 / 20 (40.0%)

*- one subject with no *H. pylori* antigens prior to eradication therapy; **- two subjects with no *H. pylori* antigens prior to eradication therapy

After successful gastric eradication, the presence of *H. pylori* antigens in the oral cavity (dental plaque and/or saliva) was found in 37.0% (10/27) of subjects; in 3 subjects *H. pylori* antigens were not found prior to the treatment. After unsuccessful gastric eradication, the presence of *H. pylori* antigens in the oral cavity was found in 40.0% (8/20) of subjects; 2 subjects had no antigens in the oral cavity before the treatment. The efficacy of gastric eradication in subjects with and without *H. pylori* antigens in the oral cavity at baseline was 63.0% (17/27) and 50.0% (10/20) ($p>0.05$), respectively. In subjects with initial *H. pylori* oral infection, the presence of *H. pylori* antigens in the oral cavity after successful and unsuccessful eradication in the stomach was 47.1% (8/17) and 50.0% (5/10) ($p>0.05$), respectively. In subjects without initial oral infection with *H. pylori*, the presence of *H. pylori* antigens in the oral cavity after successful and unsuccessful eradication therapy in the stomach was 30.0% (3/10) and 20.0% (2/10) ($p>0.05$), respectively. The efficacy of *H. pylori* eradication therapy in the dental plaque, saliva or both was 30.0%, 50.0%, and 33.3%, respectively (Table 4).

Table 4. The efficacy of *H. pylori* eradication therapy in the dental plaque, saliva or both

Dental plaque	3 / 10 (30.0%)*
Saliva	4 / 8 (50.0%)
Dental plaque and saliva	3 / 9 (33.3%)*

* - two subjects, in whom the gastric eradication was successful, but too little dental plaque was collected for the post-treatment evaluation, are not included.

DISCUSSION

Determination of *H. pylori* antigens in the oral cavity is likely encumbered with certain error. A positive result is obtained probably both for living and already dead bacteria, as well as for spiral and coccoid forms [9,14,15]. It can, therefore, be speculated that this factor was the fundamental reason for the lack of relationship between bacterial eradication in the stomach and oral cavity. Nonetheless, the different tests for *H. pylori* stomach and oral infection were used in the study; this could be a source of some divergences in the results between these two locations.

A relatively low gastric eradication rate may be attributed to the short treatment period, increasing resistance of *H. pylori* against clarithromycin, smoking habit, and consecutive but not the first attempt of eradication [16-18]. A low oral eradication rate results not only from bacterial resistance against clarithromycin, but also trace concentrations of amoxicillin in saliva and poor antibiotic penetration into the dental plaque [19-21]. Moreover, in subjects who do not brush their teeth or perform it carelessly, the bacteria that were killed or transformed into the

coccoid form during antibacterial therapy may remain in the dental plaque for many weeks, giving a positive result in the antigen test. It is of note that coccoid forms present the same set of antigens as the spiral forms [14].

The case of 5 subjects who presented with *H. pylori* antigens in the oral cavity 6 weeks after treatment, despite their absence in the pre-eradication assessment, indicates that the baseline evaluation could have been imprecise. It is less probable, yet still possible, that it was a result of new oral infection, which took place after the completion of eradication therapy. It should also be stressed that *H. pylori* may reside not only in the dental plaque and saliva, from which it is isolated most frequently, but also on the oral mucosa, periodontal pockets and palatine tonsils [15,22,23]; within these locations *H. pylori* can likely also survive eradication therapy. In the studied population with stomach infection, some subjects presented with bacterial antigens only in the saliva, some in the dental plaque and 30.0% both in the dental plaque and saliva. Large differences may result from the fact that some subjects were completely edentulous. In some, due to the lack of molars and premolars, the dental plaque was collected from the anterior teeth, which are less frequently the residence of *H. pylori* [24].

In some subjects, especially those not brushing their teeth after the evening meal, the dental plaque could have been contaminated with food. In 4 subjects, who presented with bacterial antigens in dental plaque or saliva prior to eradication therapy, the evaluation of plaque infection after treatment was impossible due to insufficient mass of the collected plaque; only the results obtained from plaques weighing over 2 mg may be considered reliable [9].

CONCLUSIONS

The immunological method detecting *H. pylori* antigens in the dental plaque and saliva cannot be recommended to evaluate the efficacy of *H. pylori* eradication in the oral cavity.

Conflicts of interest

None declared.

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The study was approved by ethics committee of the Medical University of Białystok and each subject provided written informed consent before entry to the study.

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