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ORIGINAL RESEARCH

Eradication of Gastric *Helicobacter pylori* ameliorates Halitosis and Tongue Coating

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ABSTRACT

Background: The influence of gastric *Helicobacter pylori* infection on the development of oral pathoses remains unclear. The aim of this study is to examine the influence of gastric *H. pylori* infection on occurrence of halitosis and coated tongue.

Materials and methods: Ninety-eight patients with dyspepsia were included in the study and their salivary samples and gastric biopsies were analyzed for the presence of *H. pylori* by Nested-PCR. Halitosis and coated tongue were assessed at the initial examination and 3 months after systemic eradication therapy against *H. pylori.*

Results: Gastric biopsies of 66 patients were positive for *H. pylori.* Only one saliva sample was *H. pylori* positive. At initial examination, halitosis was observed in 20 patients (30.3%) out of 66 who had gastric *H. pylori* infection and in only 3 patients (9.4%) out of 32 without *H. pylori* infection (p = 0.0236). Coated tongue was diagnosed in 18 (27.2%) patients with the infection compared to only 2 (6.25%) patients negative for gastric *H. pylori* (p = 0.0164). Patients with gastric infection were treated with the triple eradication therapy (Amoxicillin, Clarythromycin, Pantoprazol) and their gastric biopsies and oral status were examined 3 months later. Halitosis was significantly more prevalent in the group of patients with persistent *H. pylori* infection (42.1%) compared to only 6.4% of patients in the group where infection was successfully eradicated (p = 0.0012). Coated tongue was diagnosed in 47.4% of patients where *H. pylori* was still present after eradication therapy and in only 6.4% where eradication succeeded (p = 0.0003).

Conclusion: Our findings suggest that eradication of gastric *H. pylori* significantly alleviates halitosis and coated tongue, the two oral conditions that may be considered as extragastric manifestations of this common chronic bacterial infection.

Keywords: Coated tongue, Eradication therapy, Halitosis, *H. pylori.*

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INTRODUCTION

Helicobacter pylori is a microaerophylic, Gram-negative bacterium which primary ecological niche is the human stomach. *H. pylori* infection is one of the most common chronic bacterial infections worldwide, with more than half of the world's population in both developed and developing countries being infected with this organism.[4] While infection is thought to be acquired in early childhood in most cases, epidemiological studies have also suggested new infections can be acquired during adulthood. Reported annual incidence rates vary significantly and range from 0 to 35%.[8] The pathogenic properties of *H. pylori* include its ability to survive in the gastric juice, to escape the gastric acidity and to colonise the gastric mucosa. Infection with the organism is strongly associated with dyspeptic diseases (oesophagitis, peptic and duodenal ulcers, gastritis, duodenitis) and gastric cancer.[5] Current eradication regimen comprises of proton pump inhibitors and combination of antibiotics. Elimination of the infection greatly reduces the risk of disease relapse but in some cases the organism is not completely eliminated at the first attempt and the recurrence of infection is well-documented.[17]

In addition to the involvement in the local pathosis, *H. pylori* has been implicated in the pathogenesis of some extragastric diseases, such as idiopathic thrombocytopenic purpura, iron deficiency anemia, chronic idiopathic urticaria and hepatocellular carcinoma. There is also an increasing evidence for a possible association of *H. pylori* with cardiovascular diseases.[15] Pathophysiological mechanisms responsible for development of extragastic diseases include gastrointestinal blood loss and insufficient dietary iron absorption as well as autoimmune mechanisms.[9]

Halitosis and coated tongue are multifactorial oral conditions that can cause deep psychological stress to long time sufferers. A possible link between *H. pylori*

and breath odor was first suggested in 1993 and several later clinical studies reported close relationship between *H. pylori* infection, halitosis and glossitis.[1] In addition, *H. pylori* has been shown to produce hydrogen sulphide and methyl mercaptan, implying that this microorganism could contribute to the development of halitosis.[7]

The tongue coating has been shown to be a major source of volatile sulfur compounds production.[20] More than 700 bacterial species inhabit the oral cavity and the tongue coating, in particular, provides a reservoir for oral micro-organisms. Association between changes in tongue coating and gastric *H. pylori* infection has been indicated in various studies. Adler et al[1] showed association between *H. pylori* infection, burning mouth syndrome, halitosis, and lingual papillary hyperplasia while Gall-Troselj et al[6] reported connection between *H. pylori*, burning mouth syndrome and atrophic glossitis.

The detection and isolation of the *H. pylori* from the gastric mucosa have been performed successfully, however, reports of extra-gastric isolation, primarily oral cavity, are still controversial.[2] Additionally, the influence of local and gastric infections on development of oral pathology remains unclear.[3]

The aim of our study was to examine the association between gastric *H. pylori* infection, the presence of the bacterium in saliva, halitosis and coated tongue and the influence of systemic eradication therapy on these oral pathosis.

MATERIALS AND METHODS

Subjects and Sampling

In this double-blind, case-control interventional clinical study, 98 subjects with dyspepsia (42 male and 56 female, age range from 19 to 78 years) were examined. All participants signed the consent form, before undergoing research procedures, agreeing to their participation in the clinical protocol. The study was approved by the Ethical Committee of the School of Dentistry, University of Belgrade. Exclusion criteria were the use of antimicrobials and Proton Pump Inhibitors within the 3 months before the study, previous eradication therapy and hypersensitivity to amoxicillin or clarithromycin.

Two investigators assessed the presence of halitosis and the tongue coating index independently. All participants received a letter with instructions. Two days before their appointment, they had to avoid garlic, onions, and spicy food in their diet. Twelve hours before the measurements, they also had to refrain from alcohol or coffee. On the morning of the appointment, they were forbidden to use chewing gums, mints, drops, scents, and mouth rinses. In contrast, they could perform normal oral hygiene (tooth brushing) and have breakfast. All measurements were recorded between 9:00 am and 12:00 pm. The organoleptic halitosis score was determined at the distance of 10 cm by two trained judges who tested their ability to distinguish odors using the Smell Identification Test (Sensonics Inc, Haddon Heights, NJ, USA). Patients were instructed not to speak for several minutes prior to the examination. Organoleptic malodor scores[14] were recorded independently by each judge on a scale from 0 to 4 as follows: 0—no appreciable odor; 1—slight oral malodor; 2—moderate oral malodor; 3—strong oral malodor and 4—very strong oral malodor. Patients were considered positive for halitosis if both examiners scored them 2 or higher.

Tongue coating index (TCI) was assessed by visual method based on observation of thickness of tongue coating as describe by Shimizu et al.[16]

Score 0: Tongue coating not visible.

Score 1: Tongue coating thin, papillae of tongue visible.

Score 2: Tongue coating very thick, papillae of tongue not visible.

Participants were regarded as to having coated tongue if both examiners gave score 1 or higher. In addition, unstimulated saliva was collected in a sterile tube during 1 minute for *H. pylori* detection.

Subsequent to oral cavity inspection, all patients underwent upper esophagogastroduodenoscopy with biopsy, performed with GIF 165L endoscope, Olympus Co Tokyo, Japan. Two samples from the same patient were taken of the greater and lesser curvature of the gastric antrum up to 5 cm from the pylorus, and of lesion if any, and stored in a sterile tube containing 0.5 ml of Tris EDTA buffer, at –20°C until PCR analyses.

DNA Extraction and PCR Amplification

Whole DNA was extracted from saliva and gastric samples by the boiling method followed by Nested-PCR using species-specific primers for *H. pylori.* DNA from a reference strain ATCC 43629 *H. pylori* was used as a positive control. Following DNA extraction, 2 µl of DNA were used for PCR. The amplification was performed in a 25 µl mixture containing Taq DNA polymerase (1 unit/reaction), PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 7.4 and 1.5 mM $MgCl_2$), deoxynucleoside triphosphates (0.1mM/reaction of each dNTP) and primers (20 pmol each/reaction). All samples were amplified using a DNA thermal cycler (PCR Express, HYBAID Corp, USA). The cycling conditions were: initial denaturation at 94°C for 3 minutes and 35 cycles of: 94°C (1 minute), 57°C (1 minute),

72°C (1 minute) and a final extension step at 72°C for 5 minutes. The first round of amplification was performed with the following primer pair (Applied Biosystems, UK) designated as outer primers:

5′-CAGTTATTTGGTGGCTACAACCG-3′and

5′-CCCATCAATAGACGCTTAATCC-3′

The expected PCR product size was 956 bp. After the first round of PCR, 2 µl of the final product were transferred to a fresh tube and reamplified using the same program and conditions as previously indicated, but with a primer pair designated as inner primers:

5′-GCTGTAATTTAAGGGTGGGGGTTG-3′ and

5′-TGCCGTAATTCAACTGCAAGCG-3′ with an expected PCR product of 345 bp.

Polyacrylamide Gel Electrophoresis

Ten microliter of final PCR products were mixed with 2 µl of gel loading dye (0.25% bromophenol blue, 30% glycerol and 10 mM Tris EDTA, pH 7.6), loaded into 8% polyacrylamide gel and electrophoresed using 1 × TEA buffer. DNA fragments were visualized after staining with ethidium bromide using a BIO-RAD GEL-DOC 1000 detection system.

Study Design

Following the assessment of gastric *H. pylori* infection by Nested-PCR, 66 subjects positive for gastric infection were given the triple eradication therapy which consisted of Amoxicillin 2 g/day (g/d), Clarithromycin 1 g/d and Pantoprazole 80 mg/d, for 7 days. Three months after the completion of the eradication therapy all 66 participants underwent control examination with oral and endoscopic evaluation, taking samples as at the beginning of the study.

Statistical Analysis

The occurrence of halitosis and coated tongue was compared between the patients with gastric *H. pylori* infection and those without detected bacterium in their biopsy samples, both before and after the eradication therapy. Comparisons were performed using a two-tails Fisher's test. The significance was set at p-value of < 0.05.

RESULTS

At initial examination *H. pylori* was detected in gastric samples of 66 out of 98 examined subjects with dyspepsia, using Nested-PCR method. Only one saliva sample was positive for *H. pylori.* Halitosis was observed in 20 patients (30.3%) who had gastric infection and in only 3 patients (9.4%) out of 32 without *H. pylori* infection (p = 0.0236). Coated tongue was diagnosed in 18 (27.2%) patients with the infection compared to only 2 (6.25%) patients negative for gastric *H. pylori* (p = 0.0164) (Table 1).

Three months after the completion of the eradication therapy 47 (71%) patients out of originally 66 infected were negative for gastric *H. pylori* while 19 (29%) of them still harbored the bacterium in their stomach. All of the saliva samples were now negative for *H. pylori.* Halitosis was observed in 8 (42.1%) subjects with persistent gastric infection and in only 3 subjects (6.4%) from the group where infection was successfully eradicated (p = 0.0012). Coated tongue was diagnosed in 9 patients (47.4%) where *H. pylori* was still present in gastric biopsies and in only 3 patients (6.4%) where eradication therapy succeeded (p = 0.0003) (Table 2).

DISCUSSION

There is a strong correlation between *H. pylori* infection with gastritis, gastric and duodenal ulcer and the development of gastric cancer.[11] However, the association between gastric *H. pylori* infection and oral pathology is still controversial. Some studies have indicated possible involvement of *H. pylori* in the pathogenesis of glossitis, halitosis, burning mouth syndrome, recurrent aphthous ulcerations and even periodontitis.[13] Detection of *H. pylori* in oral samples varies significantly, ranging from 0 to 100%.[19] Studies evaluating prevalence of *H. pylori* in the oral cavity showed conflicting data and influence of the local infection on oral pathology is not clear.[12] The number of *H. pylori* detected in oral samples is usually very small and its presence may be only transient resulting from gastric reflux, a symptom common in gastroesophageal diseases.[2] Moreover, even if it is present in the mouth, systemic eradication therapy has been shown to have 100% eradication rate for oral *H. pylori*

Table 1: Significantly higher prevalence of halitosis and coated tongue in patients with gastric *Helicobacter pylori* infection at initial examination

Gastric samples	*Halitosis*	*Coated tongue*	*Total patients*
H. pylori +	20 (30.3%)	18 (27.2%)	66
H. pylori –	3 (9.4%)	2 (6.25%)	32
P (Fisherman's)	0.0236	0.0164	

H: Helicobacter

Table 2: Significant correlation of halitosis and coated tongue with the persistent gastric *Helicobacter pylori* infection (follow-up examination)

Gastric samples	*Halitosis*	*Coated tongue*	*Total patients*
H. pylori +	8 (41.2%)	9 (47.4%)	19
H. pylori –	3 (6.4%)	2 (6.4%)	47
P (Fisherman's)	0.0012	0.0003	

H: Helicobacter

as opposed to 78% for stomach infection.[2] In our study, *H. pylori* was detected in only one of the saliva samples taken during initial examination and in none of the samples taken after the eradication therapy. This finding rules out the influence of local *H. pylori* infection on development of halitosis and coated tongue. In addition, Kinberg et al[10] has reported that gastrointestinal pathology was very common in patients with halitosis regardless of their dental and otolaryngological findings. It has also been shown that local therapy with antiseptic mouthwashes is ineffective as a treatment of halitosis in subjects with gastric *H. pylori* infection.[17]

Our results indicate association between gastric *H. pylori* infection, halitosis and coated tongue and show for the first time that these two oral conditions are significantly improved after successful gastric eradication of this bacterium. At initial examination, about one third of infected patients suffered from halitosis and coated tongue, compared to only less than 10% of patients with dyspeptic diseases but without gastric *H. pylori* infection. Three months after completion of applied triple therapy, *H. pylori* was eradicated in 71.2% of patients. Half of the patients where eradication therapy failed and who had a recurrence of infection, still suffered from coated tongue and halitosis, while in the group where *H. pylori* was successfully eradicated the prevalence of halitosis and coated tongue was significantly lower (6%).

Several other studies have found a higher rate of halitosis among *H. pylori* positive patients with dyspeptic diseases than in those who were *H. pylori* negative.[18] Hoshi et al reported that levels of hydrogen-sulfide and dimethyl-sulfide in mouth air were significantly higher in *H. pylori* positive then in *H. pylori* negative patients.[7] In addition, gastric *H. pylori* infection has also been associated with lingual hyperplasia, burning mouth syndrome and atrophic glossitis.[22] In this interventional study, we showed for the first time that successful eradication of gastric *H. pylori* ameliorated halitosis and coated tongue.

CONCLUSION

Our findings implicate clear association between gastric *H. pylori* infection and occurrence of halitosis and coated tongue. Eradication of gastric *H. pylori* infection significantly improves these two very common oral conditions.

CLINICAL SIGNIFICANCE

In the absence of local factors, patients with persistent halitosis and coated tongue should be referred to and treated by gastroenterologists.

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