SHORT COMMUNICATION

UDC: 579.61::616.31-002.4-08
DOI: 10.2298/VSP151014113L

Eradication of *Helicobacter pylori* in patients without gastric symptoms suffering from recurrent aphthous stomatitis: A pilot study

Eradikacija *Helicobacter pylori* kod bolesnika bez gastričkih simptoma koji imaju rekurentni aftozni stomatitis: pilot studija

Marina Latković*, Lazar Ranin†, Nevenka Teodorović*, Marko Andjelković‡

University of Belgrade, Faculty of Dental Medicine, *Department of Restorative Dentistry and Endodontics, ‡Department of Prosthodontics, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, †Institute of Microbiology and Immunology, Belgrade, Serbia

Abstract

Background/Aim. *Helicobacter* (*H.*) *pylori* is a widespread bacterium and its involvement in pathogenesis of gastric diseases is well-known. However, *H. pylori* role in etiology of other histologically similar conditions, especially recurrent aphthous stomatitis (RAS) is still controversial. Research regarding *H. pylori* and its association with RAS, as well as the treatment options were always conducted on patients with diagnosed gastric problems. The aim of this study was to determine whether *H. pylori* is present in the oral cavity of patients suffering from RAS but without any symptoms or medical history of gastric disease. **Methods**. A total of 15 patients with RAS participated in the study. None of the participants suffered from any gastrointestinal disorders. Two dental plaque samples from each participant were collected. The first was analyzed using rapid urease test and the second one was put in transport medium and sent for cultivation. The sensitivity of *H. pylori* to antibiotics was established using disk diffusion method of sensitivity testing for every patient individually and adequate therapy was prescribed. **Results**. Before the treatment the mean annual recurrence rate of RAS was 8.1 ± 2.1, with the average number of lesions being 3.9 ± 1.9. During the 12-month observation period after the eradication therapy, none of the patients reported recurrence of aphthous lesions. The treatment was successful in all cases. **Conclusion**. This study shows that RAS can be effectively treated by successful eradication of oral *H. pylori*, and that RAS could be possibly considered as an early warning sign of potential gastric infection by *H. pilory*.

Key words:
stomatitis, aphthous; recurrence; helicobacter pylori; comorbidity; diagnosis; treatment outcome.

Apstrakt

Uvod/Cilj. *Helicobacter (H.) pylori* je široko rasprostanjena bakterija i njen uticaj na nastanak gastričkih oboljenja vrlo dobro je dokumentovan. Međutim, uloga *H. pylori* u patogenezi histološki sličnih oboljenja, posebno rekurentnog aftoznog stomatitisa (RAS), nije dovoljno istražena. Dosadašnje studije, u kojima je ispitivana veza između *H. pylori* i RAS, kao i moguće terapijske opcije, bile su usmerene ka bolesnicima sa prethodno dijagnostikovanim gastričkim smetnjama. Cilj ovog istraživanja bio je da se utvrdi da li je *H. pylori* prisutan u usnoj duplji i kod bolesnika bez simptoma i istorije gastričkih oboljenja koji pate od RAS. **Metode.** U studiji je učestvovalo 15 bolesnika koji pate od RAS. Bolesnici nisu imali smetnje vezane za gornji deo digestivnog trakta. Po dva uzorka dentalnog plaka prikupljena su od svakog bolesnika. Jedan plak je ispitivan uz pomoć brzog ureaza testa, dok je drugi stavljen u transportni medijum i poslat na kultivaciju. Osetljivost *H. pylori* na antibiotike određivana je uz pomoć antibiograma za svakog bolesnika posebno i, u skladu sa rezultatima, prepisivana je odgovarajuća terapija. **Rezultati.** Pre lečenja prosečan broj epizoda RAS tokom godine iznosio je 8,1 ± 2,1, sa prosečno 3,9 ± 1,9 aftoznih lezija. Tokom 12-mesečnog perioda nakon eradikacione terapije, ni kod jednog bolesnika nije došlo do ponovne pojave afti. Terapija je bila uspešna kod svih bolesnika. **Zaključak.** Rezultati ovog istraživanja pokazuju da se RAS može uspešno lečiti eradikacijom *H. pylori* i da se sama pojava RAS može posmatrati kao rano upozorenje na moguću gastričku infekciju.

Ključne reči:
stomatitis, aftozni; recidiv; helicobacter pylori; komorbiditet; dijagnoza; lečenje, ishod.

Correspondence to: Marko Andjelković**,** Faculty of Dental Medicine, Department of Prosthodontics, Rankeova 4, 11 000 Belgrade, Serbia. E-mail: markoandjelkovic@hotmail.com

Introduction

Helicobacter (*H.*) *pylori* is a widespread microaerophilic, Gram-negative, spiral bacterium associated with gastrointestinal disorders. Its involvement in pathogenesis of gastritis, gastric ulcers and malignancies is well-known [1], however, its role in etiology of other histologically similar conditions, especially recurrent aphthous stomatitis (RAS), is still controversial. Several studies tried to determine if *H. pylori* is present in aphthous lesions but the results were not very convincing [2–4]. Despite that, other authors [5, 6] suggested that *H. pylori* could be one of the important causative factors in RAS pathogenesis. Another conflicting issue is whether *H. pylori* is a resident or transient member of oral microflora, and can oral cavity act as a reservoir of these bacteria [7, 8].

Eradication treatment (triple therapy) of *H. pylori* is the therapy of choice for patients suffering from gastroduodenal diseases. It consists of two antibiotics and proton pump inhibitor, and this therapy course is supported by a wide consensus [9, 10]. Studies showed that eradication can also have positive effects on patients suffering from RAS, regarding recurrence rate, the number of lesions and severity of symptoms [5, 11]. However, this treatment may fail mostly due to *H. pylori* resistance to one of the antibiotics used [12].

Research regarding *H. pylori* and its association with RAS, as well as the treatment options were always conducted on patients with diagnosed gastric problems. In this study we tried to determine whether *H. pylori* is present in the oral cavity of patients suffering from RAS but without any symptoms or medical history implying gastric disease and can eradication, based on antibiotic sensitivity testing results, eliminate or reduce RAS symptoms.

Methods

A total of 15 patients (7 men and 8 women, aged 30 to 50) with RAS participated in the study. None of the participants suffered from any gastrointestinal disorders (dyspepsia, heartburn or peptic ulcer) and had not consumed any antibiotics at least 1 month prior to the sample collecting. Samples were collected during the active phase of RAS; diagnosis was made clinically at the time of examination at the Department of Restorative Dentistry and Endodontics, Faculty of Dental Medicine, Belgrade, Serbia.

The patients were given detailed questionnaire regarding history of aphthae appearance, their number and localization, as well as any pain and unpleasantness associated with the condition. Plaque index by Silness-Löe was measured for each patient.

Per two dental plaque subgingival samples from each participant were collected using a dental probe. The first was analyzed using rapid urease test (Bramio *H. pylori* test, The Institute for Immunology and Virology, Torlak, Belgrade, Serbia). The urease test was kept at 37°C and the result was read after 1, 2, and between 3 and 24 h; the values +++, ++, + were assigned, respectively, if the results were positive; the test was regarded positive if color had changed from yellow to red. The second sample was put into transport medium and sent for cultivation (Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia). Columbia agar (bioMerieux, France) enriched with 7% horse blood (The Institute for Immunology and Virology, Torlak, Belgrade, Serbia), 5% yeast extracts an essential amino acids was used as culture medium for cultivating *H. pylori.* Cultivation was done in microaerophilic conditions (Generbag, bioMerieux, France) at 37°C. Identification of *H. pylori* was done by typical colony (grey, circular and translucent) and microscopic characteristics (Gram-negative curved, thin bacterium), and biochemical tests.

The sensitivity of *H. pylori* to antibiotics was established using the disk diffusion method for isolates from each patient individually, and adequate therapy was prescribed. Antibiotics taken into consideration were amoxicillin, doxycycline, erythromycin, ciprofloxacin, clarithromycin and metronidazole. Treatment consisted of two antibiotics that *H. pylori* was most sensitive on, and lasted for ten days.

One month after the eradication treatment patients were recalled and control samples were obtained. Procedures for identifying *H. pylori* were repeated. Plaque index by Silness-Löe was also measured.

A control period was 12 months. During this time patients were monitored for potential recurrence of aphthous lesions.

Data were analyzed using the statistical package (SPSS version 17.01, SPSS Inc, Chicago, IL, USA). The sample size was established in 15 patients, to obtain the power higher than 80% ($\alpha = 0.05$), on the basis of the results from the study of Whitley and Ball [13]. Paired *t*-test was used to compare plaque index and χ^2-test for cultivation and rapid urease test results before and after eradication treatment. A *p*-value of < 0.05 was used to assign statistical significance for all tests. All descriptive statistics are presented as mean ± standard deviation (SD).

Results

All of the 15 patients chosen for this study completed the protocol. The average age of patients was 38 ± 6 years. Aphthous lesions were located on lower lip (20.0%), buccal mucosa (53.3%) and hard palate (26.7%).

The average plaque index by Silness-Löe at the first examination was 1.67 ± 0.20. One month after the treatment, there was no statistical difference in plaque index values (1.64 ± 0.21).

Rapid urease test was positive for all the patients, and for 13 of them the color of the test changed during the first hour (8 of them in the first 20 min). Two samples were labeled positive within the second hour. All of the colonies showed growth and were identified as *H. pylori.* After the treatment, rapid urease tests were negative for 14 patients and one test showed discrete color change after 24 h ($p < 0.05$), and there were no visible colonies in culture mediums ($p < 0.05$) (Table 1).

Before the treatment the mean annual recurrence rate was 8.1 ± 2.1, with the average number of lesions being 3.9 ± 1.9. During the 12-month follow-up after the eradication

Table 1

Results of the patients testing for *Helicobacter pylori* before and after the eradication treatment

Variable	Negative finding, n (%)	Positive finding, n (%)		
		After 1 h	After 2 h	After 3–24 h
Rapid urease test				
before treatment	0	13 (86.7)	2 (13.3)	0
after treatment	14 (93.3)	0	0	1 (6.67)
Culture				
before treatment	0			15 (100)
after treatment	15 (100)			0

therapy, none of the patients reported recurrence of aphthous lesions. The treatment was successful in all 15 patients.

Discussion

This study showed the 100% treatment success, but these results should be taken with caution due to the small sample number. Other studies reported significant accomplishments but with a fairly lower success rate [5, 14]. It can be explained by the fact that we managed to overcome bacterial resistance and improve therapy result following the results of sensitivity testing [10]. Plaque index values did not have any influence on the effect of eradication treatment.

Recent studies used modern polymerase chain reaction (PCR) methods for identifying *H. pylori* presence based on its DNA [15, 16] but design of this study demanded bacterial cultivation because only viable, active bacterial colonies could be tested on sensitivity to antibiotics. Sampling was done from gingival sulcus to obtain dental plaque because of complex biofilm structure that can provide better environment for colonization of *H. pylori* [17].

Rapid urease test was used as an additional way to indirectly confirm the presence of *H. pylori* based on its urease activity. Although there are a number of oral bacteria that can give positive test results, time in which color of test changed (within the first 2 h for 14 of the samples analyzed) suggested the presence of a microorganism with very high urease activity. A positive result in one sample after 24 h could be contributed to oral bacteria. In subgingival biofilm formations there are bacteria that can give positive rapid urease test result, but with lower urease activity that would take longer time for positive result; this indicates the presence of *H. pylori* in study participants [18].

This preliminary study shows that *H. pylori* can be found in oral environment for longer periods of time. Recurrence of aphthous lesions and their absence after *H. pylori* eradication prove a connection between this bacteria and etiology of RAS, so we can assume that it was present during the whole course of the disorder, and that oral cavity can act as a reservoir. These findings are in correlation with other studies [16, 19–21]; however, there are also authors [4, 22] with different opinions.

RAS is the most common disorder of the oral mucosa with the prevalence up to 50% in general population [23]. The cause is not entirely clear, but many factors have been considered in its etiology [24]. *H. pylori*, as stated previously, is one of the possible factors, but the exact mechanism of this microorganism contributing to RAS pathogenesis is still unclear [5, 6]. The results of this study show that successful eradication of oral *H. pylori* could treat RAS very effectively, although larger study group should be analyzed.

Knowing that *H. pylori* is among the most infectious human pathogens, infecting an estimated 50% of the global population [10], any means of preventing its propagation should be taken into consideration.

Conclusion

The fact that the patients with RAS included in this study were not suffering from gastric disorders can possibly be used as a measure of prevention and that RAS can be taken as an early warning sign of potential gastric infection by *H. pylori.*

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Received on October 14, 2015.
Revised on January 5, 2016.
Accepted on January 12, 2016.
Online First May, 2016.