Oral Diseases (2005) 11, 22–26

http://www.blackwellmunksgaard.com

Oral Medicine

Outcome following treatment for *Helicobacter pylori* in patients with recurrent aphthous stomatitis

E Albanidou-Farmaki[1], L Giannoulis[2], A Markopoulos[1], S Fotiades[3], X Aggouridaki[3], K Farmakis[4], P Papanayotou[1]

[1]*Department of Oral Medicine & Pathology, School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [2]*1st Department of Internal Medicine, AHEPA University Hospital, Thessaloniki, Greece;* [3]*Department of Immunology, AHEPA University Hospital, Thessaloniki, Greece;* [4]*2nd Department of Surgery, G. Gennimatas Hospital, Thessaloniki, Greece*

OBJECTIVE: The aim of the current study was to investigate any association of *Helicobacter Pylori* (HP) in recurrent aphthous stomatitis (RAS) and the effect of eradication of the microorganism in the clinical course of the disease.

STUDY DESIGN: Forty-eight patients with RAS were included in the study. Twenty-six were women and 22 men, of average age 41.3 ± 2.44. Thirty-four out of these 48 patients were HP positive and the rest 14 who were negative were used as a control group. The diagnosis of HP infection was based on the detection of specific immunoglobulin G (IgG), and immunoglubulin A (IgA) antibodies using the enzyme-linked immunoabsorbent assay technique in the serum and the saliva of the patients. In all HP carriers an eradication therapy was administered. After a 2-month period the patients were checked for HP status, using ^{13} C-UBT. The follow up period was 6–12 months following the eradication therapy.

RESULTS: At entry patients with HP infection suffered from more severe symptoms compared with HP negative patients ($P < 0.05$). After the administration of HP eradication therapy, patients who had become negative showed a remarkable improvement (62.5%) with reference to recurrence of RAS as well as to symptom intensity. In 29.2% of patients symptoms had disappeared and in 33.3% of patients there was a decrease in both the frequency of recurrence and the intensity of symptoms. After the eradication treatment, the periods between recurrence of RAS in patients who had become negative were statistically significantly longer compared with those before treatment ($P < 0.001$). Another important observation was that patients who became negative after eradication therapy were of comparable clinical status with those who were HP negative from the beginning of the study ($P > 0.05$).

CONCLUSIONS: These findings support the concept of a potential association between RAS and HP.
Oral Diseases (2005) **11**, 22–26

Keywords: *Helicobacter pylori*; recurrent aphthous stomatitis

Correspondence: Dr A Markopoulos, Department of Oral Medicine & Pathology, School of Dentistry, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece. Tel: +30 2310 999523, Fax: +30 2310 999455, E-mail: anmark@dent.auth.gr
Received 9 January 2003; revised 9 July 2003, 24 March 2004 and 27 April 2004, accepted 28 May 2004

Introduction

Helicobacter pylori (HP) is a microaerophilic, Gram-negative bacterium, which causes chronic active gastritis and plays a primary role in the pathogenesis of both gastric and duodenal ulcer (Lambert *et al*, 1995).

Approximately 50–70% of HP strains produce two cytotoxins: (1) VacA, whose action is enhanced by acid pH causing vacuolation and degeneration of the epithelial cells of gastric mucosa and (2) CagA, a surface protein, which constitutes the main infectious element of HP and is associated with greater HP colonization of gastroduodenal mucosa and advanced gastroduodenal disease. It possibly causes a specific T-lymphocyte response and could be a potential candidate antigen for vaccine development (Peterson and Graham, 1998; Shimoyama and Crabtree, 1998).

Helicobacter pylori has been detected in gastric secretions, faeces, saliva in the dental plaque of healthy individuals and also in patients with upper digestive system diseases (Lambert *et al*, 1995; Nguyen *et al*, 1995).

The presence of HP has been correlated with ulcerative and neoplastic diseases of the digestive system, such as, gastric lymphoma of low malignancy (MALT) and gastric cancer (De Giacomo *et al*, 1990; Mollenkopf *et al*, 1990; Strauss *et al*, 1990; Sierra *et al*, 1992; Sipponen *et al*, 1992; Blaser *et al*, 1993; Farinati *et al*, 1993; Lambert *et al*, 1995; Matsukura *et al*, 1995; Niemann *et al*, 1997; Siman *et al*, 1997; Sozzi *et al*, 1998).

These observations led us to investigate the potential role of HP in recurrent aphthous stomatitis (RAS). The data regarding the potential relationship between RAS

and HP infection are limited and conflicting (Porter and Scully, 1991; Birek *et al*, 1999; Riggio *et al*, 2000; Shimoyama *et al*, 2000; Iamaroon *et al*, 2003; Richter *et al*, 2003; Victoria *et al*, 2003).

The aim of the present study was to investigate the role of HP in the aetiology of RAS and the effect of eradication treatment of the microorganism on the clinical course of the disease.

Materials and methods

We studied 48 patients all with minor recurrent aphthous ulceration referred to the Oral Medicine Clinic. There were 26 women and 22 men, of mean age 41.3 ± 2.44 years. The clinical criteria for detection were the same in all patients and were evaluated according the number, duration and the frequency of the lesions. The diagnosis of RAS was based on clinical criteria that were proposed by Porter and Scully (1991) and Scully *et al* (1996).

Clinical assessment of RAS was based on a scale as follow: 0, no symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms. Mild symptoms were characterized the presence of one to two ulcers, whose duration was 4–7 days and their frequency of recurrence was every 2–3 months. As moderate were regarded the existence of two to five lesions with 10–15 days duration and frequency every 1 month. Severe were characterized by the presence of more than five lesions, with more than 15 days duration and continuous ulceration.

All patients were healthy and did not manifest any systemic disease. Patients on anti-secretory medication (H2 histamine antagonists and proton pump inhibitors), antibiotics and bismuth derivatives were excluded from the study. Thirty-four patients were HP positive and the rest 14 were negative. The latter were used in our study as the control group.

The diagnosis of HP infection was based on the detection of specific IgG and IgA antibodies using the enzyme-linked immunoabsorbent assay (ELISA) technique in the serum and the saliva of the patients. The presence of IgG, IgA, and CagA antibodies to HP was determined by using a commercial ELISA kit (Sorin Biomedica Diagnostics, Kasel, Germany).

The cut off values used in the study were 10 iu ml^{-1} for HP IgG, 15 iu ml^{-1} for HP IgA and 10 iu ml^{-1} for antiCagA.

Ninety-two well microtitre plates coated with HP antigens were used for the detection of anti – HP IgG & IgA in serum and saliva samples of all patients.

The HP carriers were given triple eradication treatment of omeprazole 20 mg b.i.d. plus clarithromycin 500 mg b.i.d. and amoxicillin 1 g b.i.d. for 7 days. After a 2-month period a ^{13}C-Urea INFAI test (Cufa Institute, Bochum, Germany) was performed to assess the HP infection status among the patients and was repeated at 1 and 2 years after eradication therapy (Labenz and Bosch (1994).

The follow-up period lasted 12 months with monthly visits to the clinic and included a detailed clinical assessment with particular attention to the number, the size and the recurrence rate of lesions.

Symptom scores were comparatively assessed in HP positive patients before and after eradication treatment. Clinical comparisons were also made between the HP carrier group before treatment and patients who were HP negative from the beginning, and lastly between the group of patients who became negative after treatment and the group who were HP negative from the beginning.

Statistical analysis of the results was performed using the χ^2 test and the unpaired *t*-test. χ^2 test was used for categorical analysis of positive and negative to HP patients, while *t*-unpaired test was used for the comparison of antibody values before and after treatment.

Results

On the basis of specific IgG and IgA anti-HP antibodies and anti-CagA protein, antibodies to HP were determined in the serum and the saliva of 48 patients with RAS. Thirty-four of 48 (70.8%) patients were HP positive.

As shown in Table 1, 29 patients had IgG antibodies in the serum and 12 patients had IgA antibodies. Saliva anti-HP IgA antibodies showed greater sensitivity compared with that of IgG (16 patients *vs* five patients).

In 18 patients CagA was present in serum and in three of them in saliva as well. Table 1 also presents the title range for IgG, IgA and anti-CagA antibodies in serum

Table 1 Range of the antibody titre to *Helicobacter pylori* in the serum and saliva in patients with recurrent aphthous stomatitis (RAS) and their relation with the intensity of the disease

	IgG (Cut off value = 10 iu ml^{-1})	*IgA (Cut off value = 15 iu ml^{-1})*	*CagA (Cut off value = 10 iu ml^{-1})*
Serum			
Mean value	55 ± 61 iu ml^{-1}	95 ± 37 iu ml^{-1}	47.1 ± 39.4 iu ml^{-1}
Range	14.3–137 iu ml^{-1}	18–95 iu ml^{-1}	10–110 iu ml^{-1}
No. of positive patients	29	12	18
Saliva			
Mean value	13 ± 12 iu ml^{-1}	49.7 ± 29.7 iu ml^{-1}	43.33 ± 5.77 iu ml^{-1}
Range	10–16 iu ml^{-1}	16–80 iu ml^{-1}	10–50 iu ml^{-1}
No. of positive patients	5	16	3
Intensity of RAS symptoms	Mild	Moderate	Severe

Table 2 The therapeutic effects of eradication therapy in 24 of 34 patients with recurrent aphthous stomatitis who became *Helicobacter pylori* negative

Complete cure	*Significant improvement*[a]	*Moderate improvement*[b]
Therapeutic response 7/24 (29.5%)	8/24 (33.3%)	9/24 (37.5%)

[a]When improved by two stages from severe to mild.
[b]When improved by one stage from severe to moderate or moderate to mild.

and saliva of patients with RAS and their relationship with the severity of symptoms.

After the administration of an appropriate HP eradication treatment, 15 of 24 patients became negative and showed remarkable improvement with regard to both the recurrence intervals, which were longer, and the symptom intensity of the disease. Seven of 24 patients, showed complete cure, becoming free of symptoms (29.5%), while eight patients (33.3%) demonstrated a decrease in both the frequency of the recurrence (approximately every 2 months) and symptom intensity. This improved clinical status remained stable during the whole follow-up period. However, nine out of 24 of those who had become HP negative showed moderate clinical improvement (Table 2).

Statistical evaluation before the beginning of treatment showed that patients with HP infection suffered from more severe symptoms with regard to frequency of recurrence intervals and symptom intensity compared with initially HP negative patients ($P < 0.05$) (Table 3).

It is noteworthy that after eradication treatment, the recurrence intervals in patients who had become negative were significantly longer compared with those before treatment ($P < 0.001$), a finding that suggests the beneficial effect of the treatment in the decrease of recurrences of the disease (Table 3). The intensity of the symptoms was also decreased.

Furthermore, another important observation was that the clinical status of HP positive patients, who became negative after the eradication treatment, was almost comparable with that of the 14 out the 48 patients who had been HP negative from the beginning of the study ($P > 0.05$) (Table 3).

Table 3 Comparison of patient groups with recurrent aphthous stomatitis by *Helicobacter pylori* status before and after treatment

Patients	*Interval ulcer – free (in days)*	*P*-value
Positive before treatment ($n = 34$)	31.32 + 38.62	< 0.001
Becoming negative after treatment ($n = 24$)	85.26 + 60.22	
Positive before treatment ($n = 34$)	31.32 + 38.62	< 0.05
Negative from the beginning ($n = 14$)	140.71 + 188.69	
Becoming negative after treatment ($n = 24$)	85.26 + 60.22	> 0.05
Negative from the beginning ($n = 14$)	140.71 + 188.69	

Discussion

The aetiology of RAS remains unclear. Auto-immune mechanisms or genetic factors have been implicated in its pathogenesis. In 5% of the cases it may be associated with gastrointestinal diseases (Kayavis *et al*, 1987; Albanidou-Farmaki *et al*, 1988).

Clinical observations have demonstrated a relationship between a number of gastrointestinal diseases and aphthae. In particular, oral ulceration (aphthae) has been described in patients with malabsorption syndrome or idiopathic steatorrhea, ulcerative colitis and Crohn's disease (O Mahony *et al*, 1985; Velso and Saleiro, 1987; Majorana *et al*, 1992).

Helicobacter pylori has been established to play an important role in the evolution and development of the ulcerative diseases of the upper digestive system (Blazer, 1990; NIH Consensus Conference, 1994).

This study explores the possibility of HP colonization in GI tracts of patients with RAS. In the majority of our patients with oral aphthous ulcers the presence of specific HP IgG antibodies in the serum was at a percentage of 70.8%, IgA in saliva up to 33.8%, and anti-CagA antibodies in the serum at a percentage of 37.5%. Our observations are consistent with the results of other researchers in previous studies (Birek *et al*, 1999; Riggio *et al*, 2000; Shimoyama *et al*, 2000).

Our findings also suggest that serological tests for the detection of IgG antibodies against HP are useful in the diagnosis of HP infection, while they seem to show a relation with the severity of the disease (Table 1). IgA antibodies seem to have a limited presentation in serum, but their presence in saliva is increased, a finding that is in accord with earlier studies (Sugiyama *et al*, 1995).

Our results clearly show that patients with recurrent oral ulceration appear to suffer from active HP infection in a high percentage up to (70.8%) of cases. This percentage is significantly higher; almost double than found that in healthy adults of the same age (40–50 years old) in Greece (40–50%) (Apostolopoulos *et al*, 2002). Moreover, it is remarkable that the eradication treatment of HP in patients with oral ulceration resulted in complete remission or considerable improvement of symptoms in a high percentage of patients (62.5%). This favourable result, which was maintained throughout the whole period of follow-up (12 months after the eradication treatment), was found only in patients who became HP negative after the eradication treatment. These findings may imply a possible aetiological correlation between HP infection and RAS.

The exact mechanism by which HP induces tissue injury is not clear. Immune-mediated mechanisms induced by HP have been the subject of intense investigation. HP strains have the ability to stimulate cytokine production, particularly IL-8 and to induce secretion of lymphocyte chemotactic factors with the

formation of particular T lymphocyte subpopulations. Infiltrating neutrophils can be activated by the bacterium or its extracts to produce reactive oxygen metabolites (hydrogen peroxide and hypochlorous acid) all of which are cytotoxic (Birek *et al*, 1999).

The complex aetiology of RAS including genetic, environmental, hormonal, infectious and immunologic factors has been recognized since a long time. There is a increasing evidence that focal T-cell mediated immunity (delayed type of hypersensitivity reaction or a cytotoxic response) is the mechanism ultimately responsible for tissue destruction. It is not known what particular exogenous or endogenous factors (allergens or autoantibodies) residing in the oral epithelium might trigger the immune response. However it is conceivable that in susceptible individuals mucosal changes may develop which permit the adherence of HP and subsequent production of autoantibodies to epitopes shared by oral epithelial cells and microorganisms. Furthermore, cytokine production, over expression of lymphocyte adhesion molecules and the recruitment of specific subsets of T-lymphocytes, have all been shown to play a role in oral aphthous stomatitis as they have also in HP-associated gastritis. Thus, it appears likely that HP acts at least as a co-factor in the pathogenesis of recurrent oral stomatitis, especially in individuals sensitized through gastric colonization and mucosal attachment (Bodger *et al*, 1997; Gasbarrini *et al*, 1999).

It is also possible that the patients with HP – related gastritis may have some iron and other micronutritient deficiencies that may predispose them to RAS. Thus treatment for HP may eliminate these co-factors in disease causation.

The present study does not prove an aetiological relationship between HP infection and RAS. However, it does show a strong correlation in a large subgroup (62.5%) of patients, who demonstrated a complete or considerable remission of symptoms after HP eradication treatment. This result is important, in view of the recurrent symptomatology of this disease, which negatively affects the quality of life of these patients (Llewellyn and Warnakulasuriya, 2003), and who need a range of therapeutic interventions in order to achieve clinical improvement.

In conclusion, the results of this study support the idea of a potential relationship between RAS and HP, which might or might not be aetiological. This association is seen at least in a large subgroup of patients. However, this issue still remains open and needs to be further investigated and confirmed by other controlled clinical studies.

References

Albanidou-Farmaki E, Kayavis I, Polymenidis Z, Papanagiotou P (1988). HLA-A, B, C and DR Antigens in Recurrent Oral Ulcers. *Ann Dent* **47:** 50–53.

Apostolopoulos P, Vafiadis-Zouboulis I, Tzivras M *et al* (2002). *Helicobacter pylori* infection in Greece: the changing prevalence during a ten-year period and its antigenic profile. *BMC Gastroenterol* **16:** 11.

Birek C, Grandhi R, McNeill K *et al* (1999). Detection of *Helicobacter pylori* in oral aphthous ulcers. *J Oral Pathol Med* **28:** 197–203.

Blaser MJ, Kobayashi K, Corer TL *et al* (1993). Helicobacter pylori infection in Japanese patients with adenocarcinoma of the stomach. *Int J Cancer* **55:** 799–802.

Blazer MJ (1990). *Helicobacter pylori* and the pathogenesis of gastroduodenal inflammation. *J Infect Dis* **161:** 626–633.

Bodger K, Wyatt JI, Heatley RV (1997). Gastric mucosal secretion of interleukin-10: relations to histopathology, *Helicobacter pylori* status, and tumor necrosis factor-a secretion. *Gut* **40:** 739–744.

De Giacomo C, Fiocca R, Villani L *et al* (1990). *Helicobacter pylori* infection and chronic gastritis: clinical, serological and histologic correlations in children treated with amoxicillin and colloidalbismuth subcitrate. *J Pediatr Gastroenterol Nutr* **11:** 310–316.

Farinati F, Valiante F, Germana G *et al* (1993). Prevalence of *Helicobacter pylori* infection in patients with precancerous changes and gastric cancer. *Eur J Cancer Prev* **2:** 321–326.

Gasbarrini A, Franceschi F, Arnuzzi A (1999). Extradigestive manifestations of Helicobacter pylori infection. *Gut* (Suppl I): 1–12.

Iamaroon A, Chaimano S, Linpisarn S *et al* (2003). Detection of *Helicobacter pylori* in recurrent aphthous ulceration by nested PCR. *J Oral Sci* **45:** 107–110.

Kayavis I, Daniilidis M, Albanidou-Farmaki E *et al* (1987). T-Lymphocyte subsets in recurrent oral ulceration. A preliminary study. *J Oral Med* **42:** 198–201.

Labenz J, Bosch G (1994). High significant change of the clinical course of relapsing and complicated Peptic ulcer disease after cure of *Helicobacter Pylori* infection. *Am J Gastroenterol* **89:** 1785–1788.

Lambert JR, Lin SK, Aranda-Michel J (1995). Helicobacter pylori. *Scand J Gastroenterol* **30**(Suppl. 208)**:** 22–46.

Llewellyn CD, Warnakulasuriya S (2003). The impact of stomatological disease on oral health-related quality of life. *Eur J Oral Sci* **111:** 297–304

Majorana A, Sapelli PL, Malagoli A *et al* (1992). Celiac disease and recurrent aphthous stomatitis; the clinical and immunogenetic aspects. *Minerva Stomatol* **41:** 33–40.

Matsukura N, Onda M, Yamashita K (1995). *Helicobacter pylori* in peptic ulcer and gastric cancer. *Gam To Kagaku Ryoho* **22:** 169–178.

Mollenkopf C, Steininger H, Weineck G, Meyer M (1990). Gastritis immunohistochemical detection of specific and nonspecific immune response to *Helicobacter pylori*. *Z Gastroenterol* **28:** 327–334.

Nguyen AMH, EI Zaatari FAK, Graham DV (1995). *Helicobacter pylori* in the oral cavity. *Oral Surg Oral Med Oral Radiol Endod* **76:** 705–709.

Niemann T, Larsen S, Mouritsen EA, Thorsgaard N (1997). *Helicobacter pylori* infection in patients with chronic pancreatitis and duodenal ulcer. *Scand J Gastroenterol* **32:** 1201–1203.

NIH Consensus Conference (1994). *Helicobacter pylori* in peptic ulcer disease. Consensus development panel on *Helicobacter pylori* in peptic ulcer disease. *JAMA* **272:** 65–69.

O Mahony C, O Farrelly C, Weir DG *et al* (1985). Gluten sensitive oral ulceration in the absence of coeliac disease. *Gut* **26:** A1137.

Peterson WL, Graham DY (1998). *Helicobacter pylori*. In: *Sleisenger and fordrans gastrointestinal and liver disease*. WB Saunders: Philadelphia.

Porter SR, Scully C (1991). Aphthous stomatitis: an overview of etiopathogenesis and management. *Clin Exp Dermatol* **16:** 235–243.

Richter J, Grimmova M, Stiborova I *et al* (2003). Detection of *Helicobacter pylori* in the saliva of patients with recurrent aphthous stomatitis. *Cas Lek Cesk* **142:** 665–669.

Riggio MP, Lennon A, Wray D (2000). Detection of *Helicobacter pylori* DNA in recurrent aphthous stomatitis tissue by PCR. *J Oral Pathol Med* **10:** 507–513.

Scully C, Flint S, Porter SR (1996). *Oral diseases*. Martin Dunitz: London, pp. 1–371.

Shimoyama T, Crabtree JE (1998). Bacterial factors and immune pathogenesis in *Helicobacter pylori* infection. *Gut* **43**(Suppl. 1)**:** S2.

Shimoyama T, Horie N, Kato T, Kaneko T, Komiyama K (2000). *Helicobacter pylori* in oral ulcerations. *J Oral Sci* **42:** 225–229.

Sierra R, Munoz N, Pena AS *et al* (1992). Antibodies to *Helicobacter pylori* and pepsinogen levels in children from Costa Rica: comparison of two areas with different risks for stomach cancer. *Cancer Epidemiol Biomarkers Prev* **1:** 449–454.

Siman JH, Forsgren A, Berglund G, Floren GH (1997). Association between *Helicobacter pylori* and gastric carcinoma in the city of Malmo, Sweden. A prospective study. *Scand J Gastroenterol* **32:** 1215–1221.

Sipponen P, Kosnnen TV, Valle J *et al* (1992). *Helicobacter pylori* infection and chronic gastritis in gastric cancer. *J Clin Pathol* **45:** 319–323.

Sozzi M, Valentini M, Figura N *et al* (1998). Atrophic gastritis and intestinal metaplasia in *Helicobacter pylori* infection: the role of CagA status. *Am J Gastroenteroleogy* **93:** 375–329.

Strauss RM, Wang TC, Kelsey PB *et al* (1990). Association of *Helicobacter pylori* infection with dyspeptic symptoms in patients undergoing gastroduodenoscopy. *Am J Med* **89:** 464–469.

Sugiyama T, Yabana T, Yachi A (1995). Mucosal immune response to *Helicobacter pylori* and cytotoxic mechanism. *Scand J Gastroenterol* **208**(Suppl.)**:** 30–32.

Velso FT, Saleiro JV (1987). Small bowel changes in recurrent ulceration of the mouth. *Hepatogastroenterology* **34:** 36–37.

Victoria JM, Kalapothakis E, Silva J, Gomez RS (2003). *Helicobacter pylori* DNA in recurrent aphthous stomatitis. *J Oral Pathol Med* **32:** 219–223.