Helicobacter pylori in oral ulcerations

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(Received 2 August and accepted 13 December 2000)

Abstract: Helicobacter pylori is an important pathogen involved in the development of gastrointestinal ulcers, but its involvement in oral ulcerous lesions is unclear. As culture is generally recognized as the gold standard for diagnosis of H. pylori infection, we employed this approach to assess the association of H. pylori with oral mucosal ulcerations. Samples were collected from patients with oral mucosal ulcerative disorders: 12 cases of recurrent aphthous stomatitis (RAS), 7 cases of herpes simplex virus (HSV) stomatitis, and 3 cases of erosive lichen planus (LP). Serum IgG antibodies against H. pylori were examined in all cases. All of the RAS and erosive LP cases were culture-negative for H. pylori, while two cases of HSV stomatitis were positive. The two culture-positive cases were also seropositive for the H. pylori antigen. It has been suggested that the oral cavity might be an alternative reservoir for H. pylori (11,12). Recently, an association of H. pylori with oral ulcerative disorders has been proposed (13,14). Birek et al. (15) detected H. pylori in recurrent oral aphthous ulcers by using PCR assay. Data pertinent to the relationship between H. pylori and oral ulceration are limited, and no study using culture methods has been reported. As yet it has not been clear whether H. pylori is a pathogen involved in the development of oral ulcerative disorders. In this study, we used culture to assess the association of H. pylori with oral ulcerous lesions, and in addition, we examined serum IgG antibodies against H. pylori.

Key words: Helicobacter pylori; culture; recurrent aphthous stomatitis; lichen planus; herpes simplex virus.

Introduction

Helicobacter pylori was first isolated from a human gastric biopsy specimen in 1984 (1). Today, it is considered to be a pathogen important to the development of gastrointestinal ulcers and it has been implicated as a carcinogen for gastric cancer and mucosa-associated lymphoid-tissue lymphoma of the stomach (2, 3). H. pylori has been detected in dental plaque, saliva, and the subgingival region (4-8), and there have been reports that H. pylori strains in the mouth and stomach are identical (7,9,10). It has been suggested that the oral cavity might exist in a non-culturable coccoid state without productive infection, and might form colonies only under special conditions such as HSV infection. (J. Oral Sci. 42, 225-229 , 2000)

Materials and Methods

Twenty-two (9 male, 13 female) participants with oral ulceration were selected from patients visiting the Oral Surgery Clinic of Saitama Medical Center. The disorders consisted of 12 cases of recurrent aphthous stomatitis (RAS), 7 cases of herpes simplex virus (HSV) stomatitis, and 3 cases of erosive lichen planus (LP) (Table 1). Only patients with no known history of peptic ulceration or other upper gastrointestinal disorders and no symptoms of upper gastrointestinal disease were included. None of the patients was immunosuppressed nor had had prior treatment for oral ulcers. The diagnosis of RAS was based on accepted clinical criteria (16). The diagnosis of HSV infection was determined by serum examination (17). The diagnosis of LP was made histopathologically by biopsy.
All patients gave informed consent in accordance with the guidelines of the Committee on Human Investigation. The oral samples were collected for the culture test by swabbing the ulcer surfaces with sterile cotton swabs. The samples were cultured on a selective medium for H. pylori (Selective Media HP, Eiken, Tokyo) which contained calf heart brain extract (450 g), peptone (10 g), glucose (2 g), NaCl (5 g), Na_2,HPO_4 (2.5 g), vancomycin (10 mg), polymyxin B (2500 units), trimethoprim (5 mg), amphotericin B (2 mg), agar (15 g), and defibrinated horse blood (70 ml per 1000 ml). Media were adjusted to pH 7.2 and cultures were grown under microaerophilic conditions at 37 °C for 3-7 days (19). Identification of H. pylori was based on colony morphology, Gram staining, motility, and positive oxidase, catalase and urease tests (4).

Serum IgG antibodies against H. pylori in all participants were also measured by an in-house enzyme-linked immunosorbent assay technique using the Helico-G serum ELISA kit (Porton Cambridge, Newmarket, UK), which detects the human anti-H. pylori IgG against total cell lysates of H. pylori (20,21). This assay system has been reported to have 91.2% sensitivity and 69.0% specificity in Japan, in comparison with the culture test (22). The analysis was carried out according to the manufacturer's instructions. Serum (100 μl) diluted 1:200 with diluent was incubated in microwells precoated with H. pylori antigen for 60 min at 37°C. Plates were then washed seven times with diluent, and 100 ml peroxidase-conjugated F(ab')2 fragment of goat anti-human IgG was added to each well. After incubation for 30 min at 37°C the wells were washed seven times. A solution (100 μl) of substrate (including 3,3',5,5'-tetramethylbenzidine 0.24 mg/ml) was added to each well. After incubation at room temperature for 10 min in the dark, the reaction was stopped with 50 μl stop solution, 1.5 N sulfuric acid, in each well. Absorbance was read at 450 nm using a microelisa plate reader. The manufacturer's recommended cut-off value of 10 U/ml (optical density of 0.330) was used to define a patient's serology as H. pylori-positive or H. pylori-negative.

### Results

Serum IgG levels of the 22 patients ranged from 0.049 to 1.475 (mean ± SD: 0.373 ± 0.371). Nine patients (40.9 %) were serum IgG-positive (range 0.353 to 1.475, 0.720 ± 0.349) and 13 were negative (range 0.049 to 0.286, 0.132 ± 0.078). The results of the culture and detection of serum IgG antibodies against H. pylori are summarized in Table 2. None of 12 patients with RAS were positive for culture. Three of 12 cases were H. pylori-seropositive and 9 of 12 cases were seronegative. None of the three patients with erosive LP was positive for culture. One of the three was seropositive. Two of seven with HSV stomatitis were positive for culture. Both the two culture-positive cases were seropositive for H. pylori. Of the five culture-negative cases, four were seronegative. The serum IgG levels of these culture-positive cases were 0.714 and 0.428.

### Discussion

Culture is the gold standard for diagnosis of H. pylori infection (2,23), but it is very unlikely that oral H. pylori would be detected by culture (4,6,9). A low number of organisms or loss of viability during processing of dental specimens might contribute the poor sensitivity of culture (24, 25). Ishihara et al. (19) reported that various oral bacterial species inhibited the growth of H. pylori by producing bacteriocin-like inhibitory proteins. Aiba et al. (26) reported that Lactobacillus salivarius was able to produce a large amount of lactic acid and therefore completely inhibit the growth of H. pylori. Bode et al. (27) contended that H. pylori could convert into a basally respiring but nonculturable coccoid state under physical or chemical stress. H. pylori in the oral cavity would invariably exist in coccoid form.

The infection rate of H. pylori in an asymptomatic population, determined by serological methods, has been reported to be approximately 40-70 % (22,28) in Japan and 10-40% in other developed countries (29). It has been suggested that the risk factors for H. pylori infection are sanitary conditions during childhood (25,30). Porter et al. (14) reported that the frequency of anti-H. pylori seropositivity was not significantly elevated among patients with RAS and other ulcerative or nonulcerative oral mucosal disorders. In our study, 9 of the 22 patients were seropositive (40.1 %), and the ratio of positive to negative seemed to be essentially no different in comparison with that for asymptomatic controls. It is suggested that the relationship between H. pylori and oral ulceration cannot
be drawn from the serological method alone.

Histologically, a similarity between gastrointestinal ulcer and oral aphthous ulcer has been suggested (15). Leimola-Virtanen et al. (13), using in situ hybridization, found H. pylori DNA in 6 of 29 (20.7%) oral mucosal ulcers of immunocompetent patients. Birek et al. (15) reported that 23 of 32 (71.9%) of recurrent aphthous ulcers detected by PCR assay were positive for H. pylori and suggested a relationship between H. pylori and recurrent aphthous ulcers. On the other hand, Mravak-Stipetic et al. (31), using nested PCR, suggested that H. pylori was not pathogenic in the oral cavity, nor was it associated with a common oral pathogenic process because H. pylori was rarely present in ulcerous and nonulcerous oral cavity lesions. In our study, H. pylori could not be detected by culture in any RAS patients, whether the patient was seropositive or not for the H. pylori antigen. Our results support the view that H. pylori might not be a pathogen involved in RAS. But the microbiology of dental plaque is a complex of numerous fragile and fastidious forms. Estimates that 300 or more species reside in subgingival sites appear to be realistic and perhaps even conservative (32). H. pylori, even though a trigger of the ulcer formation, may be thrust aside and inhibited in growth by commensal microorganisms in the mouth during the ulcer formation. Accumulation of more cases might be needed to clarify whether H. pylori is related to the ulceration of RAS.

All erosive LP were also negative on culture. Mravak-Stipetic et al. (31), who used in situ hybridization, reported that H. pylori DNA was detected in 4 of 21 (19%) cases of oral LP, but the percentage of positive patients with ulcer and nonulcer disease was almost the same. They reported that there was no relationship between H. pylori and LP ulceration. The histological features of LP are definitely different from those of gastric ulcer. We suspect that there may be no relationship between H. pylori and LP ulceration. Two of seven cases of HSV stomatitis were positive on culture. Both cases were seropositive for H. pylori. In addition, seronegative cases were culture-negative. H. pylori does not appear to be an etiologic factor for HSV stomatitis. Mravak-Stipetic et al. (31) indicated that mucosal change might make the environment more acceptable for H. pylori than the normal mucosa. Though reflux from the stomach could not be ignored (33), H. pylori that already existed in the oral cavity would multiply and would be detected according to the alteration of the oral environment and oral microflora provoked by the HSV infection.

In this study H. pylori might not be associated with oral ulcerative disorders. However, it is a fact that H. pylori has been detected in various dental samples, and there have been reports that paired strains from the mouth and stomach were identical (7,9). Oshowo et al. (7) reported that oral colonization was a rare event, but did occur. Probably, H. pylori in the oral cavity exist in the nonculturable coccoid state without a productive infection and could be colonizing only under special conditions, such as with HSV infection.

References

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