Prevalence of *Helicobacter pylori* in benign gastric ulcers in a cohort of Sri Lankan patients

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Abstract

Helicobacter pylori prevalence is decreasing globally and prevalence of non *H. pylori* gastric ulcers is increasing. The following study was conducted to assess the prevalence of *H. pylori* in benign gastric ulcers in a sample of Sri Lankan patients. This was a cross-sectional study of 59 dyspeptic patients with benign gastric ulcers. Multiple endoscopic gastric biopsies were obtained and histology, immunohistochemistry and polymerase chain reaction were performed for *H. pylori* detection. An immunochromatography assay was performed to detect blood anti *H. pylori* antibodies. Four (6.8%) were positive for *H. pylori*. Therefore, it is likely that most benign gastric ulcers are of non-*H. pylori* aetiology.

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Introduction

With the description of a curved rod shaped bacterium (*Helicobacter pylori*) in a benign gastric ulcer in 1980s there was much enthusiasm to investigate the role of *H. pylori* in the pathogenesis of gastro-duodenal diseases [1]. Early studies have indicated 70 to 90% prevalence of *H. pylori* infection in benign gastric ulcers and 90 to 100% in duodenal ulcers [2]. However, more recent studies have indicated that the association between *H. pylori* infection and peptic ulcer disease is not as strong as indicated in previous literature [3-6]. The prevalence of *H. pylori* infection is coming down globally and this has been observed particularly in developed countries [7]. On the other hand, proportion of non-steroidal anti-inflammatory drugs (NSAIDs) related peptic ulceration and non-NSAID, non-*H. pylori* ulcers are increasing in Western as well as in some developed Asian countries [3-6]. These observations indicate that the role of *H. pylori* in the pathogenesis of peptic ulcer disease is decreasing.

There are only a few studies on aetiological association of benign gastric ulcers from Sri Lanka. Fernando et al. reported a *H. pylori* prevalence of 59% and 58% in duodenal and gastric ulcers respectively using only CLO test, and Waidyarathne *et.al* reported a prevalence of 51.8% in gastric ulcers using only histology alone [8, 9]. We conducted the present study to assess the prevalence of *H. pylori* infection in benign gastric ulcers in a cohort of Sri Lankan patients, using histology, immunohistochemistry, polymerase chain reaction and serology as *H. pylori* detection methods.

Methods

This was a cross-sectional study of dyspeptic patients undergoing upper gastrointestinal endoscopy (UGIE) from March 2012 to August 2013, at the Teaching Hospital, Peradeniya, Sri Lanka. Patients who were detected to have benign gastric ulcers, confirmed with histology, were selected for analysis. Mucosal breaches more than 3 mm in diameter with some depth were regarded as ulcers and superficial smaller breaches which were classified as erosions were excluded [10]. Multiple endoscopic biopsies were obtained from the ulcer edge, antrum, insicura angularis and the body from each patient. Histology based haematoxylin and eosin stain and toluidine blue stain, immunohistochemistry and polymerase chain reaction (PCR) were performed on gastric biopsies of every patient to detect *H. pylori* organisms. Venous blood was drawn from each patient to assess anti *H. pylori* antibodies. Positivity with any one of the test methods was regarded as evidence of *H. pylori* infection.

Biopsies for histology and immunohistochemistry were collected in 10% formaldehyde and the biopsy for PCR was collected fresh and stored at -80^{o}C. Immuno-histochemical staining was performed using indirect immunoperoxidase method (DAKO B0471 anti *H. pylori* antibodies) on formalin fixed paraffin embedded

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tissue. PCR was performed using primer HP1/HP2 for 16SrRNA locus. Serum anti *H. pylori* antibodies were assessed using a qualitative immunochromatography based assay (SD BIOLINE). This method collectively detects anti *H. pylori* antibodies and cannot differentiate the antibody sub- types; the test has a sensitivity of 95.5% and specificity of 89.6%, according to the manufacturer. Accordingly, this test was used as a screening method to detect anti *H. pylori* antibodies.

As quality control measures, positive controls were run with immunohistochemistry, PCR and immunochromatography methods and for PCR, in addition, tests were performed in duplicates.

Approval for the study was obtained from the Ethical Review Committee of Faculty of Medicine, University of Peradeniya.

Results

A total of 656 dyspeptic patients underwent UGIE during the study period and of them 59 (8.4%) had benign gastric ulcers. The mean age of the patients with ulcers was 62 ± 14.2 years (range 22 to 89 years) and 35 (59.3%) were males. Fifty six ulcers were in the antrum and the rest in the proximal stomach. Almost all the ulcers were less than 1 cm in diameter and none exceeded 2 cm. Twenty nine ulcers were associated with erosions elsewhere in the gastric mucosa and three were associated with duodenal ulcers and two with duodenal erosions. Twenty two had a history of haematemesis, melaena or both.

One showed positivity for *H. pylori* with all the test methods used, and three were positive with PCR only. Overall, four cases (6.8%) showed positivity for *H. pylori* with at least one of the test methods used. All *H. pylori* positive ulcers were present in the gastric antrum (two in the pylorus, one in the incissura and one in the rest of the antrum) and all had associated erosions. Duodenum was unremarkable in all cases. The mean age of *H. pylori* positive cases was 69.8 ± 19 years and in *H. pylori* negative cases 61.4 ± 13.8 years (the difference was not statistically significant). Three patients with *H pylori* positive gastric ulcers were men.

Discussion

In the studied population with gastric ulcers, only 6.8% (n=4) had evidence of *H. pylori* infection and the majority (93.2%) had no evidence of *H. pylori* infection with any of the test methods used. Seroprevalence of anti-*H.pylori* antibodies was 1.7%, which indicates low exposure rate to *H. pylori* in the study population. Furthermore, this very low seroprevalence excludes the possibility of false negative results due to previous exposure to antibiotics.

Western literature shows that with the declining role of *H. pylori* in the pathogenesis of gastric ulcers, drug related causes, especially NSAIDs and low dose aspirin have gained importance [3-6]. Furthermore, prevalence of non NSAID non *H. pylori* gastric ulcers is known to be increasing and the aetiology of this group of ulcers is still not clear [3-6]. The role of bile reflux in the pathogenesis of these ulcers is not known because of the difficulty in assessing the degree of bile reflux. Although diseases such as Zollinger-Ellison syndrome, hyperparathyroidism and gastric Crohn disease are known to cause gastric ulcers, these diseases are not common enough to explain the relatively high prevalence of non-NSAID non-*H. pylori* gastric ulcers. Psychological and physical stress, cigarette smoking and genetic predisposition are other contributory factors of gastric ulcers.

Under-recognition of non-*H. pylori* causes of gastric ulcers could lead to unnecessary treatment of patients with *H. pylori* eradication therapy and ulcer recurrence. Therefore, it is time to shift our focus from *H. pylori* and consider other possible causes for gastric ulcers.

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Conflicts of interests

There are no conflicts of interest.

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