Isolation and Characterization of *Helicobacter pylori* Strains from Peptic Ulcer Patients in Dhaka, Bangladesh

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Abstract

To study the association of *Helicobacter pylori* with peptic ulcer and the associated histopathological changes, to characterize the isolated strains in terms of their protein profile, 38 peptic ulcer cases were studied. A high association of *H. pylori* with peptic ulcer (duodenal ulcer 77%, gastric ulcer 75%) and gastritis (74%) was observed. Age and smoking did not have any relationship with *H. pylori* infection. The infection was predominantly associated with the 'quiescent' form of chronic gastritis. Comparative sodium dodecyl sulfate polyacrylamide gel electrophoresis of whole cell extracts of the local isolates and a reference strain from Australia showed a general homogeneity between the strains with obvious interstrain differences. However, the difference between the local isolates and the reference strain was more marked. Significant association of *H. pylori* with peptic ulcer along with strain variations were observed.


Key words: *Helicobacter pylori*, peptic ulcer, gastritis, protein profile.

Introduction

Since the first report on isolation of *Helicobacter pylori* by Marshall in 1983, this organism has been identified as a cause of chronic gastritis and as one of the important associations of peptic ulcer. There have been many reports on *H. pylori* infection from developing countries. In different geographical areas, *H. pylori* infection shows different histopathological manifestations. Although peptic ulcer is a common ailment in Bangladesh there is no report from this area on the prevalence and manifestation of *H. pylori* infection. The present study was carried out to investigate the association of *H. pylori* with peptic ulcer and to find out the *H. pylori*-associated histopathological changes. The isolated strains were characterized in terms of their protein profile and compared with a reference strain.

Methods

Study population: Patients attending the endoscopy clinic of a teaching hospital and having endoscopic findings of peptic ulcer were taken up for the study. Informed consent was obtained from all. Patients with evidence of any other local or systemic disease or history of intake of corticosteroids, non-steroidal anti-inflammatory drugs or antimicrobial agents in the preceding four weeks were excluded.

On endoscopy, three antral biopsies were obtained from intact mucosa within 3 cm of the pylorus. Patients were classified as duodenal ulcer (DU) or gastric ulcer (GU). In the last eight subjects with DU two additional biopsies were taken from intact mucosa around the ulcer site. The samples for culture were collected in 0.5 mL of 20% glucose solution and for histopathology in 10% formalin solution.

Bacterial culture: The biopsy samples were minced and streaked on chocolate agar medium containing brain-heart infusion agar base and 7% sheep blood supplemented with 1% Isovitalex (BBL Microbiology Systems, Cockeysville, MD, USA), vancomycin (6 µg/mL), nalidixic acid (20 µg/mL), trimethoprim (5 µg/mL) and amphotericin B (2 µg/mL). *H. pylori* reference strain (NCTC 11638), obtained through the courtesy of Dr. Goodwin of Royal Perth Hospital, Australia, was also cultured. The plates were incubated under microaerophilic condition (Campy Pak, BBL Microbiology Systems) for 5 to 7 days. The bacteria were identified by colony character, Gram stain, oxidase, catalase and urease tests.

Histology: For histological study, formalin-fixed paraffin embedded sections were stained with hematoxylin and eosin, examined under light microscope and classified according to Whitehead et al. Increased presence of mononuclear cells was considered as evidence of chronic gastritis; it was considered 'active' if there was infiltration...
with neutrophil polymorphs also in superficial epithelium and ‘quiescent’ if there were only few or no cells.

**Preparation of whole cell extracts:** Bacteria, grown on chocolate agar media, were harvested in sterile distilled water and were washed twice at 10,000 X g for 15 minutes at 4°C. The pellet was suspended in distilled water and was adjusted to an optical density of 0.5 at 546 nm; 15 mL of this suspension was centrifuged at 800 g for 30 minutes and the pellet was resuspended in 0.5 mL of distilled water. Protein concentration of the suspension was measured using a standard technique for microquantitation of protein. The suspension was stored at -20°C until used.

**Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE):**

The protein profile of whole cell preparations was analyzed by vertical SDS-PAGE system using a stacking gel of 4.5% and a separating gel of 13.5% acrylamide. Each well was filled with 50 μg of protein and electrophoresis was carried out overnight at 8 mAmp. The gels were stained with Coomassie brilliant blue R-250 (Sigma Chemical Co, St Louis, Mo, USA) and destained with several changes of 5% methanol and 7.5% acetic acid.

**Results**

Eighty three consecutive subjects with peptic ulcer were included in the study; these included 61 (67.3%) with DU, 15 (13.5%) with GU and 6 (6.6%) with concomitant DU and GU.

Culture of the antral biopsy specimens yielded *H. pylori* from 47/61 (77%) patients with DU, 12/16 GU and 3/6 with DU and GU. Culture of the duodenal biopsy specimens, taken from intact mucosa around the ulcer, yielded the organism in 6 of 8 cases.

The influence of age and smoking on *H. pylori* infection in DU cases was analyzed. The difference in isolation rates between different age groups was not significant. In duodenal ulcer patients, *H. pylori* was isolated from 19/28 (67.8%) non-smokers and 16/19 (84.2%) smokers. This difference was also not significant.

Of the 61 patients, (with DU) antral biopsies of 43 were subjected to histological examination. Forty two of those 43 cases showed chronic gastritis, 32 (76.2%) of them were quiescent and the remaining active. *H. pylori* was isolated from 31/42 (73.8%) cases with chronic gastritis, including 8/10 with ‘active gastritis and 23/32 (71.9%) with ‘quiescent’ gastritis. The difference was not significant.

The protein profile of the local strains isolated from the peptic ulcer cases was compared with that of the NCTC 11638 reference strain isolated from a peptic ulcer case in Australia and a *Campylobacter jejuni* isolate. A total of eight local isolates were studied.

Seven major intensity bands of molecular masses 65, 58, 51-52, 30, 26, 17 and 16 kilodalton (Kd) were found to be shared by all the local isolates and the NCTC 11638 strain (Fig). Besides these, a major band of molecular mass 63 Kd was shared by two of the local isolates and the reference strain (lanes A, D and E). That band was either missing or present in very low intensity in the other local isolates. A major band of molecular weight 45 Kd, present in the NCTC 11638 strain, was absent in the local isolates. As compared to the seven major intensity bands of *H. pylori*, only three major bands having molecular masses of 44 Kd and 16 Kd were observed in the local *C. jejuni* isolate. The 44 Kd band was, however, the most prominent.

**Discussion**

*H. pylori* isolation rates of 75% and 77% from antral biopsy samples of GD and DU cases respectively in our study are consistent with those from other countries (59-98% and 77-100%, respectively) and differ from those of Nair et al and Nanivadekar et al from India, who observed an insignificant association of *H. pylori* with peptic ulcer. Use of relatively insensitive culture media and presence of ‘quiescent’ gastritis in all patients under study may have led to the low isolation rates in these studies. In our study the isolation rates could be improved further by properly gaining the tissue and avoid-
ing antibacterial agents like naldixic acid. Duodenal colonization rates of *H. pylori* in DU patients is also in conformity with other studies.  

There was no significant difference in the isolation rate of *H. pylori* from peptic ulcer patients in different age groups. This is in conformity with the observation of lack of association of peptic ulcer with age even though the frequency of *H. pylori* infection among the general population rises with each decade.

In our patients 'quiescent' form of chronic gastritis (76.22%) was a more predominant manifestation of *H. pylori* infection as compared to the 'active' form (23.8%). Similar trend also has been reported in studies from India. Nanivadekar reported 'quiescent' gastritis in all *H. pylori*-infected cases. Maira and Ghose observed predominantly 'quiescent' gastritis in *H. pylori*-infected patients. This is different from the chronic active form of *H. pylori*-associated gastritis reported from other areas. The exact reason underlying this difference is not clear; possible reasons include earlier onset of *H. pylori* infection, strain differences, genetic factors, et al. Long history of improved public health services could also influence the manifestation of *H. pylori* infection.

SDS-PAGE profile showed that many protein bands were shared between the investigated isolates and the control strain. However, there were obvious differences between the strains. The differences between the local isolates and the NCTC 11638 were, however, more prominent. The significance of this finding is not clear. Variations in protein profile of the organism, reflecting strain differences, also may explain differences in gastric histological response.

References