Antibodies to Cag A protein are not predictive of serious gastroduodenal disease in Indian patients

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Objective: The present study was aimed at assessing the predictive utility of anti-Cag A antibodies in differentiating patients of duodenal ulcer (DU) and non ulcer dyspepsia (NUD) from asymptomatic controls in a developing country. Methods: Sera from 120 subjects were tested for antibodies to Cag A using the Immunodominant portion of a recombinant 37.5 kDa fusion protein by ELISA, in endoscopically proven cases of DU and NUD and healthy controls. Results: The observed optical density (OD) in DU and NUD patients was 1.947 and 1.960 respectively, which was higher than that observed in controls (p<0.01), but there was no difference in the anti-Cag A antibody titers between DU and NUD patients. Conclusion: Anti-Cag A antibodies do not seem to discriminate duodenal ulcer patients from non ulcer dyspepsia in the Indian population. [Indian J Gastroenterol 1998; 17: 126-128]

Key words: Acid-peptic disease, Helicobacter pylori, peptic ulcer

Helicobacter pylori infects more than 50% of the world’s human population; in developing countries the rate of infection may be as high as 90%.1 However, only a minority (<20%) of the population develops “serious” gastroduodenal disease such as peptic ulcer, gastric adenocarcinoma and MALT lymphoma, and this has been related to infection with “ulcerogen” and “carcinogenic” strains.2-4 Of the various virulence factors of H. pylori that have been studied in relation to the development of peptic ulcer and gastric cancer is the cytotoxin-associated gene (Cag A) protein, a 120-140 kDa protein that has been shown to be highly immunogenic and linked to symptomatic gastroduodenal disease, particularly in developed countries.5,6

See editorial on page 123

Serological studies have shown a high prevalence of anti-Cag A antibodies in patients with duodenal ulcer (DU) and gastric cancer,7 and several studies have been carried out to establish the role of this protein in pathogenesis of gastroduodenal disease.8-11

Studies linking the carriage of Cag A+ strains of H. pylori and specific disease states have emerged predominantly from symptomatic patients in developed countries; this relationship has not been evaluated in developing countries like India, nor has the prevalence of Cag A+ strains in asymptomatic controls been assessed.

Excellent correlation between the occurrence of DU and serum anti-Cag A antibody levels has been shown in Italian subjects.12 Duodenal and gastric ulcer patients in Hong Kong have higher anti-Cag A antibody titers than healthy H. pylori-positive controls.13

We examined the seroprevalence of antibody to a recombinant fragment of the Cag A protein in patients with DU, non ulcer dyspepsia (NUD) with or without antral gastritis, and asymptomatic controls (N) in a developing country where the prevalence of H. pylori infection is much higher than in the West. This would provide information on the role of Cag A+ strains in India and permit evaluation of a serodiagnostic test based on this antibody.

Methods

A cohort of 120 subjects was included in the study, comprising 76 H. pylori-positive dyspeptic patients (defined as having upper abdominal pain or discomfort with or without vomiting) attending the Ulcer Clinic of the Institute and 44 asymptomatic healthy controls (blood bank donors). All dyspeptic patients underwent a routine upper gastrointestinal endoscopy and were labeled as DU active (duodenal ulcer) or NUD (no ulcer in the duodenum, no deformity of the duodenal bulb, with or without antral gastritis). Three antral biopsies from each patient were tested for H. pylori by a previously validated in-house rapid urea test (using 3% modified Christensen’s urea solution), histology (using hematoxylin and cosin/Giemsia stain) and culture (Mueller-Hinton agar). At endoscopy, 50 patients had active DU with antral gastritis and 26 had only antral gastritis. The patients labeled as NUD were earlier evaluated for gallstones and worm infestation and found to be negative. Control subjects did not undergo endoscopy. Sera were collected from patients and controls and stored at -20°C till testing.

ELISA

Cag A antigen: 10 µL of the sera diluted 1:10 was used. A recombinant fusion protein of 37.5 kDa coding for the immunodominant portion of the Cag A protein of H. pylori (obtained from Dr Rino Rappuoli, Immunobiological Research Institute, Siena, Italy) was used to test for antibodies by ELISA.

The method of Xiang et al12 was followed. Briefly, purified recombinant antigen was diluted in 0.1M carbon-
ate buffer (pH 9.6) to a final concentration of 7.5 μg/ml. Flat bottomed polystyrene microtiter plates (Dynatech, India) were coated with 100 μL/well of antigen solution and incubated at 4°C overnight, washed (x 3) with PBS containing Tween 20 (PBS-T) (0.05%) and blocked with 200 μL/well of 1% BSA in PBS-T at 37°C for 2 h. They were then washed (x 3) with PBS-T and incubated with 100 μL/well of goat antihuman IgG-HRP conjugate (Bangalore Genei, India), diluted 1:1000 in blocking buffer, at 37°C for 90 min. After repeated washing (x 3) 100 μL of the substrate solution containing tetramethyl benzidine/hydrogen peroxide (TMB/H₂O₂) was added to each well. Each plate contained a positive and negative control serum as well as an antigen control. After 15 min of incubation at room temperature in the dark, the reaction was stopped using 100 μL/well of 1N H₂SO₄, and the color developed was read at 450 nm in a micro ELISA reader.

**Statistical analysis**

Sensitivity, specificity and predictive values were calculated for the assay using standard formulae. One-way analysis of variance was applied to detect the significance between the mean values. χ² test was used to test for statistical significance of difference between values.

**Results**

The study population included 50 patients with DU (38 men; mean age 30 years, range 22-68), 26 with NUD (14 men; 38 years, 26-67) and 44 asymptomatic controls (29 men; 40 years, 32-65). Their mean (SD) optical density (OD) values of anti-Cag A antibody assay were 1.947 (0.711), 1.960 (0.687) and 1.267 (0.278), respectively; the OD in the controls was significantly lower (p<0.01) than in the patients. When the cut-off was taken as mean + 1 SD of the control group (1.545), the antibody titer was positive in 64 samples: 36 (72%) patients with DU, 16 (61.5%) with NUD and 12 (27%) controls.

The sensitivity and specificity of the test were 68.4% and 72.7%, respectively. It had a positive predictive value of 81.3% and a negative predictive value of 57.1%. A receiver operator characteristic (ROC) curve was drawn (Fig) to summarize the sensitivity and specificity for the test using arbitrary cut-off scores.

**Discussion**

While a number of studies from the West have reported a significant association of Cag A-positive H. pylori strains with peptic ulcer as compared to NUD and healthy controls, there are no studies on this phenomenon from developing countries like India. Several unanswered questions — whether Cag A+ strains predict occurrence of DU, whether these antibody titers decrease after therapy — necessitate a study of anti-Cag A antibodies in various subgroups of patients in a developing country.

Xiang et al., using sera from Italian patients, found that antibodies to Cag A provide excellent correlation with the diagnosis of DU. Similar results have been reported from Australia. However, Ching et al. from Hong Kong reported that, when the same test was applied to sera from Chinese patients, the discrimination was much less and the presence of antibodies was not predictable for gastric cancer.

In the present study, the titers in the patients with DU were not significantly different from those in the NUD patients, indicating that antibodies to Cag A would not serve the purpose of identifying patients suffering from active DU among dyspeptics. The titers were significantly higher in patients with gastritis and DU when compared to normal controls. This finding is similar to that reported from Australian patients and also from the United Kingdom.

We believe that the occurrence of Cag A+ strains-related “serious” gastroesophageal disease (viz. active ulceration) is a phenomenon typical to particular geographical areas only, and there is a significant geographic variation in the prevalence of Cag A+ strains, and their role in causation of DU. This viewpoint has been reported by others, who have shown that the cagA gene is present in a majority of H. pylori strains independent of whether the individual has DU or asymptomatic gastritis. The outcome of H. pylori infection is thus a result of interactions between several factors, including acid secretion by the stomach, virulence of the organism and the nutritional status of the individual from childhood.

We also found, interestingly, that the mean OD value obtained in healthy volunteers in our study was much higher than that reported by Dr Rappuoli (personal communication) and elsewhere using the same recombinant antigen. The presence of high baseline titers of Cag A antibodies may be an indication of high or early prevalence of Cag A-positive strains in the stomachs of our population without causation of “serious” gastroesophageal disease.
The sensitivity and specificity of the test depends on the prevalence of *H. pylori* in the control population being tested and will thus vary in different geographical settings. One of the ways to express the relationship between the sensitivity and specificity of a test is to construct an ROC curve: tests that discriminate the best are placed towards the upper left corner of the curve, and the overall accuracy can be described by the area under the curve. The ROC curve shows that the recombinant Cag A fragment does not serve as a good antigen to discriminate the degree of inflammation in the Indian population (Fig).

We conclude that though there was a significant difference in the titers observed between healthy individuals and patients with NUD and DU, it was not possible to differentiate between patients with NUD and endoscopic gastritis from those with active DU and endoscopic gastritis. A number of other putative virulence factors together with host factors, may play a role in our cases.

References


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