

Serum IgG response to differentiated antigens of *Helicobacter pylori*

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Abstract

AIM To detect antibodies against *Helicobacter pylori* spiral and coccoid antigens in human sera.

METHODS Blood samples were collected from 278 patients with gastric diseases. A 3-day-old culture of *H. pylori* on chocolate blood agar was used to provide spiral form. 'Synchronous' coccoids were cultured in (BHY) (brain heart infusion supplemented with 10% horse serum and 0.4% yeast extract) medium in a chemostat. Antigens from spiral and coccoid form were prepared using acid glycine extraction. Enzyme-linked immunosorbent assay (ELISA) was performed to detect serum IgG antibodies against spiral and coccoid forms of *H. pylori*.

RESULTS Seroprevalence of *H. pylori* infection was higher in patients with gastric ulcer (79%) and gastric cancer (83%) than those with non-ulcer dyspepsia (NUD) (44%) and other diseases (45%) ($P < 0.05$). IgG antibodies against spiral and coccoid antigens were detected in 50.7% (141/278) and 49.6% (138/278), respectively.

CONCLUSION The spiral and coccoid forms of *H. pylori* coexist in patients infected with the bacterium.

INTRODUCTION

It was reported that *Helicobacter pylori* can convert to coccoid form from spiral form *in vitro* after prolonged incubation^[1] or under antibiotic stress^[2]. By histological examination, Chan *et al*^[3] and Janas *et al*^[4] observed two morphological forms of the bacteria in *H. pylori*-positive gastric specimens. The dimorphism of *H. pylori* has stimulated researchers to investigate whether coccoid form of *H. pylori* is viable and pathogenic^[1,5,6]. Coccoid form like its spiral form has similar proteins except that a high-molecular-mass antigenic fraction (>94kDa), absent in spiral forms, was detected during coccoid conversion^[6] and that a smaller number of immunoreactive bands were recognized in the coccoid antigens compared with the spiral antigens^[5]. In this study, we used ELISA technique to detect immune response of IgG to spiral and coccoid antigens.

MATERIALS AND METHODS

Serum specimens

Blood samples were collected from 278 patients with gastric disorders at the National University Hospital, Singapore. Informed consent was obtained from all the patients. After collection, blood specimens were allowed to clot at room temperature for 36mins-60mins. The sera were removed from the clot and any remaining insoluble material removed by centrifugation at 2 000×g for 10min at 4°C. Sera were stored at -20°C until use.

Antigen preparation

A local *H. pylori* strain V2 isolated from a patient with non-ulcer dyspepsia was used. A 3-day-old culture of *H. pylori* on chocolate blood agar was used to provide spiral form. 'Synchronous' coccoids were cultured in BHY (brain heart infusion supplemented with 10% horse serum and 0.4% yeast extract) medium in a chemostat. Antigens from spiral and coccoid form were prepared according to a modified method of Goodwin *et al*^[7] as described by Vijayakumari *et al*^[5].

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed according to the method described previously^[8]. Briefly, flat-bottomed microtitre plates (Nunc) were coated with acid

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glycine extract antigens of *H. pylori*. The sera tested were diluted at 1:100. Each diluted serum was examined in triplicate. Positive control serum was diluted at 1:100, 1:200, 1:400, 1:800, 1:1 600 and 1:3 200 and negative control serum at 1:100. Horse radish peroxidase-labelled rabbit anti-human IgG (Dako) was used as conjugates. The substrate used was ophenylenediamine dihydrochloride (OPD, Sigma). The optical density at wavelength 490 and 620nm reference filter was read immediately using an ELISA reader (Ceres 900 Bio-Tek Instruments, Inc). The cut-off value for the ELISA was derived according to Khin and Ho^[8].

RESULTS

Seroprevalence of *H. pylori* infection was higher in patients with gastric ulcer (79%) and gastric cancer (83%) than those with non-ulcer dyspepsia (NUD) (44%) and other diseases (45%) ($P<0.05$). Furthermore, of the 278 sera, IgG antibodies against NCTC 11637 spiral and coccoid antigens were detected in 141 (50.7%) cases and 138 (49.6%) cases.

In 191 NUD patients, 84 (43.9%) and 86 (45.0%) were detected with IgG antibodies to spiral and coccoid antigens, respectively. There was no significant difference in seroreactivity to spiral and coccoid antigens in patients with NUD ($P>0.05$) (Figure 1).

Of the 47 peptic ulcer patients, 37 (78.7%) and 33 (70.2%) showed antibodies IgG against spiral and coccoid antigens ($P>0.05$). Among the peptic ulcer patients, 69.2% (9/13) and 76.9% (10/13) of the gastric ulcer patients while 82.1% (23/28) and 67.9% (19/28) were positive for spiral and coccoid antigens, respectively. Serum IgG antibodies against spiral antigens were detected in 83.3% (5/6) patients with both gastric and duodenal ulcer, while 66.7% (4/6) of these were seropositive for IgG antibodies against coccoid antigens (Figure 1).

Of the 6 cases of gastric cancer antibodies IgG 5 (83.3%) cases were positive with IgG antibodies to spiral antigens and 2 (33%) cases were positive with IgG antibodies to coccoid antigens. No statistical difference was found in IgG antibodies against spiral and coccoid for antigens in this group ($P>0.05$) (Figure 1).

Of the 34 cases of other diseases, there was 45.4% (15/33) and 51.5% (17/33) had positive seroreactivity against *H. pylori* spiral and coccoid antigens respectively. There was no significant difference ($P>0.05$) (Figure 1).

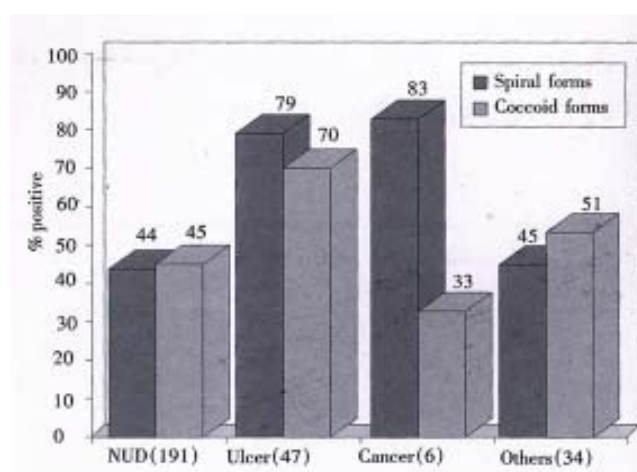


Figure 1 Prevalence of antibodies against *H. pylori* spiral and coccoid antigens in patients with different gastric diseases.

DISCUSSION

Most adult patients colonized with *H. pylori* elicit a measurable systemic antibody response which comprises predominantly IgG. In this study, the systemic immune response of 278 sera was examined by ELISA. IgG to *H. pylori* spiral and coccoid antigens was found to be 50.7% and 49.6%, respectively. The serological response to both spiral and coccoid antigens indicates the possibility of coexistence of two forms of *H. pylori* in these patients which leads to the production of similar IgG responses in patients. It has been shown earlier that there were differences in protein profiles of spiral and coccoid antigens^[6]. *in vitro*, coccoid form could be detected 6 hours after exposure to 10ng/L of amoxycillin^[2]. Janas *et al*^[4] reported the presence of two different morphological forms of *H. pylori* in gastric antrum specimens. Could these two morphological forms of spiral and coccoid of *H. pylori* be the complete cell cycle *in vivo*? The morphological changes could have been triggered under physical or chemical stress which is unfavourable to *H. pylori*. Spiral form may then convert into coccoid form. When the environment becomes favourable, the bacteria may revert from coccoid form to spiral form *in vivo*. However, what could have led to the revision is still unknown. Some *in vitro* studies indicated that coccoid form of *H. pylori* might be viable^[1,9-11]. Furthermore, Vijayakumari *et al*^[5] showed that the adherence patterns of coccoid form of *H. pylori* on kato III *in vitro* were similar to those observed with spiral form in gastric biopsy specimens *in vivo*. It was reported that the vital cytotoxic proteins of spiral forms were also conserved in the coccoids^[6]. Therefore, like its counterpart, coccoid form could be an infective, transmissible, immunogenic and pathogenic form of *H. pylori* which participates in

the pathogenesis of gastroduodenal diseases.

The coccoid form was present above damaged epithelial cells^[4] and is not easy to be detected by the techniques now being used. Patients with morphological conversion of *H. pylori* from spiral to coccoid form after treatment may be neglected and considered as eradication of *H. pylori*. The possible cell cycle of *H. pylori* may have clinical relevance. Xia *et al*^[12] reported that recurrence of *H. pylori* infection was probably caused by recrudescence in the patients studied. One possible reason of relapse could be morphological reversion of *H. pylori* from coccoid form to spiral form after treatment.

This study demonstrated the presence of antibodies to different antigens of *H. pylori* in patients. Coccoid form like its counterpart spiral form may have clinical relevance in the outcome of gastric diseases.

REFERENCES

- 1 Hua J, Ho B. Is the coccoid form of *Helicobacter pylori* viable *Microbioscience*, 1996;87:103-112
- 2 Berry V, Jennings K, Woodnutt G. Bactericidal and morphological effects of Amoxicillin on *Helicobacter pylori*. *Antimicrob Agents Chemother*, 1995;39:1859-1861
- 3 Chan W. Y., Hui P. K., Leung K. M. and Thomas T. M. M. Modes of *Helicobacter* colonization and gastric epithelial damage. *Histopathology*, 1992;21:521-528
- 4 Jans B, Czkwianianc E, Bak Romaniszyn L, Bartel H, Tosik D, Ptaneta-Malecka I. Electro microscopic study of association between coccoid forms of *Helicobacter pylori* and gastric epithelial cells. *Am J Gastroenterol*, 1995;90:1829-1833
- 5 Vijayakumari S, Khin MM, Jiang B, Ho B. The pathogenic role of the coccoid form of *Helicobacter pylori*. *Cytobioscience*, 1995;82:251-260
- 6 Benaissa M, Babin P, Quillard N, Pezennec L, Cenatiempo Y, Fauchere JL. Changes in *Helicobacter pylori* ultrastructure and antigens during conversion from the bacillary to the coccoid form. *Infect Immun*, 1996;64:2331-2335
- 7 Goodwin CS, Blincow E, Peterson G, Sanderson C, Cheng W, Marshall BJ, Warren JR, McCulloch R. Enzyme-linked immunosorbent assay for *Campylobacter pyloridis*: correlation with presence of *C. pyloridis* in the gastric mucosa. *J Infect Dis*, 1987;155:488-494
- 8 Khin MM, Ho B. Immunological detection of *Helicobacter pylori* pregnant woman. *Biomedical Letters*, 1994;52:71-78
- 9 Mai U, Geis G, Leying H, Ruhl G, Opferkuch S. Dimorphism of *Campylobacter pylori*. In: Megraud F, Lamouliatte H, eds. *Gastroduodenal pathology and Campylobacter pylori*. Amsterdam: Elsevier Science, 1989:29-33
- 10 Bode G, Mauch F, Malfertheiner P. The coccoid forms of *Helicobacter pylori*. Criteria for their viability. *Epidemiol Infect*, 1993;111:483-490
- 11 Sorber M, Nilsson M, Hanberger H, Nilsson LE. Morphologic conversion of *Helicobacter pylori* from bacillary to coccoid form. *Eur J Clin Microbiol Infect Dis*, 1996;15:216-221
- 12 Xia HX, Windle HJ, Marshall DG, Smyth CJ, Keane CT, O'Morain CA. Recrudescence of *Helicobacter pylori* after apparently successful eradication: novel application of randomly amplified polymorphic DNA fingerprinting. *Gut*, 1995;37:30-34