

Atrophic gastritis and *Helicobacter pylori* infection in outpatients referred for gastroscopy

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Abstract

Background—Atrophic gastritis has been shown to be one of the long term sequelae of *Helicobacter pylori* infection.

Aims—To determine the prevalence of atrophic gastritis in outpatients, to study the accuracy of serological methods for revealing atrophy, and to define the association of *H pylori* infection with atrophic gastritis in these patients.

Patients/methods—A total of 207 consecutive outpatients referred for gastroscopy were included. Biopsy specimens from the antrum and corpus were assessed histologically according to the Sydney system. Serum samples were studied for *H pylori* IgG and IgA antibodies by enzyme immunoassay, CagA antibodies by immunoblot, pepsinogen I by an immunoenzymometric assay, gastrin by radioimmunoassay, and parietal cell antibodies by indirect immunofluorescence.

Results—Histological examination revealed atrophic gastritis in 52 (25%) of 207 patients. *H pylori* and CagA antibodies were strongly associated with atrophic antral gastritis but poorly associated with atrophic corpus gastritis. Low serum pepsinogen I was the most sensitive and specific indicator of moderate and severe atrophic corpus gastritis. All six patients with moderate atrophic corpus gastritis had *H pylori* infection but eight of 10 patients with severe atrophic corpus had increased parietal cell antibodies and nine had no signs of *H pylori* infection.

Conclusions—Atrophic antral gastritis was strongly associated with CagA positive *H pylori* infection. Severe atrophic corpus gastritis was not determined by *H pylori* tests but low serum pepsinogen I, high gastrin, and parietal cell antibodies may be valuable in detecting these changes.

(Gut 2000;46:460–463)

Keywords: *Helicobacter pylori*; atrophic gastritis; CagA antibodies; *Helicobacter pylori* antibodies; pepsinogen; parietal cell antibodies

Gastric atrophy was first recognised in 1870 in postmortem samples of a patient with pernicious anaemia,¹ and thereafter corpus dominant atrophic autoimmune gastritis associated with pernicious anaemia has become a well known condition.² Since the development of fibregastroscopy techniques, the gastric mucosa has become easier to study in living subjects. The recognition and culture of

Helicobacter pylori (*H pylori*) has largely changed the understanding of the aetiology of gastritis³ and atrophic gastritis has been shown to be a consequence of long term gastritis caused by *H pylori*.⁴ However, gastric autoantibodies have also been demonstrated in subjects with *H pylori* positive gastritis,⁵ and a question has arisen of whether or not *H pylori* also plays a role in the aetiology of autoimmune type atrophic gastritis.⁶

Atrophic changes of the corpus can be determined by blood tests, such as low serum pepsinogen I, high serum gastrin, and parietal cell antibodies.⁷ In the present study, different serological markers of atrophic gastritis and *H pylori* infection were measured in 207 consecutive patients who underwent gastroscopy at an outpatient clinic. The objectives of the study were to determine the prevalence of atrophic gastritis in outpatients referred for endoscopy, to study the accuracy of different serological methods in revealing atrophic gastritis in these patients, and to determine to which type of atrophic gastritis *H pylori* infection is associated.

Patients and methods

PATIENTS

A total of 207 consecutive adult outpatients (age range 19–83 years; median 55 years; 122 women) referred to the Helsinki Municipal Hospital at Herattoniemi for upper endoscopy between October 1996 and March 1997 were included. Patients previously participated in a study evaluating a new *H pylori* rapid diagnostic test.⁸ Only those who had not had prior helicobacter eradication therapy were included.

ENDOSCOPY AND HISTOLOGY

Endoscopies were performed by two of the authors (AO, LV). Two biopsy specimens for histological examination were obtained from the antrum and the corpus (both the anterior and posterior walls of each). The biopsy specimens were stained with haematoxylin-eosin, Alcian blue (pH 2.5)-periodic acid-Schiff, and modified Giemsa stains. The specimens were examined in a blinded manner by a pathologist (PS) and scored in accordance with the Sydney system.⁹ The presence of *H pylori*, chronic and acute gastritis, atrophy (loss of proper glands), and intestinal metaplasia were scored 0–3 (none, mild, moderate, or severe) for the antrum and corpus separately.

Abbreviations used in this paper: *H pylori*, *Helicobacter pylori*; EIA, enzyme immunoassay; IgG, IgG; immunoglobulins G and A.

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Accepted for publication 20 October 1999

Table 1 Number of patients with atrophic gastritis and positive results in the following tests: elevated *H pylori* antibodies of IgG and/or IgA class (*H pylori* antibodies), low serum pepsinogen I level (<28 µg/ml; Low Peps I), high serum gastrin level (>111 pg/ml; High gastrin), parietal cell antibodies (PCA) and CagA antibodies (CagA). Atrophy was graded as none (0), mild (1), moderate (2), or severe (3)

	Atrophy grade	<i>H pylori</i> antibodies	Low Peps I	High gastrin	PCA	CagA	Total
Antrum atrophy	0	58	11	14	14	49	171
	1	22	4	6	3	20	30
	2	4	0	1	0	4	4
	3	2	0	0	0	2	2
Corpus atrophy	0	69	1	6	7	62	179
	1	10	1	2	1	7	11
	2	6	3	2	1	4	6
	3	0	10	10	8	1	10

SEROLOGICAL TESTS

Blood was obtained from each patient during the visit to the endoscopy unit. Separated serum samples were stored at -20°C until analysed. Immunoglobulin G (IgG) and IgA antibodies to *H pylori* were measured separately using an inhouse enzyme immunoassay (EIA).¹⁰ The antigen used was an acid glycine extract from *H pylori* strain NCTC 11637. Absorbance readings were converted to reciprocals of the end point titres. The end point titres were dilutions of serum at the cut off level defined by the optical densities of positive reference serum pools at constant dilutions. Separate reference pools were used for IgG and IgA. Serum gastrin concentrations were measured using a radioimmunoassay kit (ICN Pharmaceuticals, Diagnostics Division, USA). The antibody of the kit recognises the major circulating forms of gastrin. According to the manufacturer's instructions, the expected normal range of gastrin in healthy volunteers aged 19–60 years is 25–111 pg/ml (mean 49.6 pg/ml). Serum pepsinogen I concentrations were measured using an immunoenzymometric assay (Gastroset PG1, Orion Diagnostica, Espoo, Finland). The expected range for pepsinogen I in healthy volunteers is 28–158 µg/l (mean 84.4 µg/l).

CagA antibodies were determined using an immunoblot method. CagA antigen was prepared from NCTC 11637 strain by harvesting the bacteria in Tris HCl buffer 10 mM in a stock solution of 7.7 µg protein/µl. Bacterial suspension was run in a 7.5% SDS-PAGE gel. Proteins were transferred to a nitrocellulose membrane at 4°C overnight using a blotting device. The CagA protein band was confirmed using a specific rabbit anti-CagA antibody (a gift from Dr Martin Blaser). Strips were incubated with serum samples, diluted 1:100. After washing, they were treated with antihuman IgG conjugated horseradish peroxidase (Dako, Glostrup, Denmark) and the substrate diaminobenzidine. Positive bands were estimated visually. CagA antibodies were tested blindly.

Parietal cell antibodies were studied using an indirect immunofluorescence technique with unfixed cryostat sections (5 µm thick) of a tissue block as substrate, as previously described.¹¹ The block contained the following tissues: mouse stomach, rat stomach, rat kidney, and mouse liver. Sera were screened at 1:10 dilutions. Sera staining mouse parietal cells were regarded as positive and titrated fur-

ther. The last dilution at which unequivocal staining was seen was taken as the end point titre.

STATISTICAL ANALYSIS

Fisher's exact test was used to compare the proportions of different characteristics between groups, and p values <0.05 were considered statistically significant. The Mann-Whitney exact test was used to compare histological features between CagA positive and negative groups. Calculations were performed using StatXact 3 for Windows (Statistical Software for Exact Nonparametric Inference; Cytel Software Corporation 1995, Cambridge Massachusetts, USA).

Results

A total of 52 patients (age range 34–83 years; median age 67 years; 33 (63%) women) had atrophic gastritis. In 24 patients, atrophic gastritis was seen only in the antrum, in 16 patients only in the corpus, and in 11 patients both areas were affected. Corpus biopsies were not available for one patient with moderate atrophic antral gastritis. Of the 27 patients with atrophic corpus gastritis, 16 patients had intestinal metaplasia in the corpus also (the grade of metaplasia being the same (six patients), or one (nine patients) or two (one patient) grades lower than that of the atrophy grade). All 36 patients with atrophic antral gastritis had intestinal metaplasia which, in 35 cases was the same grade as that of atrophy.

Seventy seven patients had helicobacters on histological examination and 31 of these (40%; age range 38–83 years; median age 66 years; 21 women) had atrophic gastritis in the antrum or in the corpus, while only 21 (16%; age range 38–82 years; median age 68 years; 12 women) of the 130 histologically helicobacter negative patients had any atrophic changes (p=0.0002). Of the 31 *H pylori* positive patients with atrophic gastritis, atrophic changes (mild or moderate) were seen in the antrum only in 17 patients. In five patients, atrophic gastritis (mild or moderate) was detected in the corpus only whereas in eight patients, including two patients with severe atrophic antral gastritis and intestinal metaplasia, atrophic changes were seen in both the antrum and corpus. Six patients (age range 54–83 years; median age 67 years) with histologically detected *H pylori* had moderate atrophic gastritis in the corpus. Table 1 gives the number of patients with different grades of atrophic changes. Atrophic antral gastritis was found significantly (p=0.0001) more often in *H pylori* positive than in *H pylori* negative patients irrespective of whether or not the infection was verified by histology or by increased *H pylori* antibodies. Of the 21 histologically helicobacter negative patients with atrophic changes, five patients with increased *H pylori* IgG antibodies had only mild atrophic gastritis.

Severe atrophic corpus gastritis (total loss of normal oxyntic glands and parietal cells) was detected in 10 patients (age range 50–82 years; median age 70 years; five women). They showed no histological or serological evidence

Table 2 Sensitivity (Sens), specificity (Spec), and positive (PPV) and negative (NPV) predictive values of tests for the detection of moderate (grade 2) and severe (grade 3) atrophic corpus gastritis in 207 consecutive adult patients referred for gastroscopy

	%Sens	%Spec	%PPV	%NPV
Serum pepsinogen I <28 µg/l	81	99	87	98
Serum gastrin >111 pg/ml	75	96	60	98
Parietal cell antibodies	56	96	53	96
<i>H pylori</i> antibodies (IgG and/or IgA)	38	58	8	92
CagA antibodies	31	64	7	92

of *H pylori* infection, except for one patient with a positive CagA result (table 1). In six of these patients, the antrum showed normal histology. All 10 patients with severe atrophic corpus gastritis (table 1) had low serum pepsinogen I values (range 5–19 µg/l) and high serum gastrin levels (range 361–1097 pg/ml; upper normal limit 111 pg/ml), and parietal cell antibodies were present (antibody titre range 250–6251; median 750) in eight (five women, three men). Vitamin B12 levels were below the lower limit of normal in nine of 10 patients. In addition, parietal cell antibodies were detected in nine more patients (antibody titre range 50–6251; median 250), all women, eight of whom had helicobacters on histological examination and six had no atrophy; one patient showed normal gastric histology. Parietal cell antibodies were associated with female sex both in the whole study group ($p=0.0431$) and in the *H pylori* positive patient group ($p=0.0198$). In addition, parietal cell antibodies were significantly associated with the presence of atrophic gastritis in all 207 patients ($p=0.0004$) but not in the helicobacter positive group. Table 2 shows the accuracy of the different serological tests in detecting severe or moderate atrophic corpus gastritis. Low serum gastrin levels were detected in 20 patients, 16 of whom had normal gastric histology.

Eleven patients had atrophic changes in the antrum and corpus. Three patients with severe atrophic corpus gastritis had mild atrophic changes in the antrum. None of these patients showed signs of *H pylori* infection whereas two had parietal cell antibodies. All of the eight more patients with atrophic changes in both the antrum and corpus had helicobacters on histological examination and none had parietal cell antibodies. Two patients had severe atrophic antrum gastritis and moderate or mild atrophic changes in the corpus; one had moderate atrophic antrum gastritis and mild atrophic changes in the corpus, and two had moderate atrophic corpus gastritis with only mild atrophic changes in the antrum. Three more patients had only mild atrophic changes in both the antrum and corpus.

CagA antibodies were found in 61 (79%) helicobacter positive patients on histology. In addition, five histologically helicobacter negative patients with elevated *H pylori* IgG antibodies had positive CagA serology. Furthermore, nine patients with neither helicobacters on histological examination nor elevated *H pylori* antibodies on EIA showed positive CagA serology. Of the latter, five had normal gastric histology. Among helicobacter positive patients determined by histology, atrophic antral gastritis was more common in

CagA positive than in CagA negative subjects ($p=0.0462$) but no such association was found between CagA positivity and atrophic changes in the corpus ($p=0.8178$). Furthermore, CagA positive patients had higher scores for polymorphonuclear ($p=0.0164$) but not for mononuclear infiltration ($p=0.0575$) in the antrum. No such association was found between CagA positivity and inflammatory scores of the corpus or with helicobacter density in the antrum or corpus. All 12 duodenal ulcer patients had positive CagA serology, but of the five histologically helicobacter positive patients with gastric ulcers, only three showed CagA antibodies.

Discussion

In agreement with previous studies,^{4,12} atrophic gastritis was more common in helicobacter positive than negative patients in our study group. However, as atrophic changes in the corpus and antrum were measured separately, *H pylori* positivity, verified either by serology or histology, was strongly associated only with atrophic antral gastritis. Only after exclusion of 10 patients with severe atrophic corpus gastritis was *H pylori* infection associated with atrophic changes in the corpus also.

The relationship between *H pylori* infection and severe atrophic corpus gastritis with or without pernicious anaemia is debatable. In many studies, patients with pernicious anaemia rarely, if ever, have serological or histological signs of *H pylori* infection.^{13–15} In a Swedish study, however, 83% of patients with pernicious anaemia had antibodies against *H pylori*.¹⁶ Before the era of *H pylori*, first degree relatives of patients with pernicious anaemia were shown to have more corpus but not antrum atrophy than controls.¹⁷ Even if atrophic antral gastritis is regarded as a direct result of *H pylori* infection, factors other than *H pylori* could play a role in the development of severe atrophic corpus gastritis and pernicious anaemia. More recently it has been shown¹⁸ that in patients without pernicious anaemia (normal vitamin B12 levels) the prevalence of *H pylori* antibodies was lower in those with more severe atrophy probably because of the disappearance of *H pylori* infection after progression of atrophy and hypochlorhydria.

In our 10 patients with severe atrophic corpus gastritis, there were no signs of current or past *H pylori* infection, with the exception of one patient with positive CagA serology. At least two of the following three tests were positive in all 10 patients: low pepsinogen I, high gastrin level, and parietal cell antibodies. These patients appeared to form a distinct group and to differ from those six *H pylori* positive patients with moderate atrophic corpus gastritis, although some of the latter also had low pepsinogen I and high gastrin levels. There was no significant difference in the median ages of these two groups, as would be expected if severe atrophic corpus gastritis were regarded solely as an end stage of the progressing *H pylori* gastritis.

H pylori infection was shown to be associated with atrophic corpus gastritis only after exclusion of 10 patients with *H pylori* negative

severe atrophic corpus gastritis. Thus a large number of patients with pernicious anaemia may mask the association of *H pylori* gastritis and atrophic corpus gastritis. This may be especially so in Northern European countries where the prevalence of pernicious anaemia, probably of genetic origin,¹⁹ is considerably higher than in many other countries.²⁰ In a Finnish random sample representing the population of Southern Finland, 1.4% of individuals had severe atrophic corpus gastritis with no atrophic changes in the antrum, whereas 11% had atrophic gastritis in the antrum only or in both the antrum and corpus.²¹ In our study, the prevalence of severe atrophic corpus gastritis was considerably higher than in the random sample material. This is understandable as our study included some patients who had been referred for gastroscopy because of low serum vitamin B12 levels.

cagA positive *H pylori* strains have been shown to cause more severe gastritis than *cagA* negative strains,²² and patients infected with *cagA* positive strains may also be more susceptible to peptic ulcers.²³ Gastric cancer^{24 25} is more common in those infected with *cagA* positive strains. It has been shown that atrophic corpus gastritis develops more often in *CagA* positive than in *CagA* negative patients.²⁶ In addition, a clear correlation between atrophic antral gastritis and *CagA* seropositivity has been demonstrated.²⁷ In the present study, among helicobacter positive patients, *CagA* positivity was not associated with atrophic gastritis in the corpus but was associated with atrophic antral gastritis. In agreement with our results, *CagA* seropositivity correlated with atrophic antral gastritis but not with atrophic corpus gastritis in an earlier UK study.²⁸ It is uncertain why in our study nine patients with neither helicobacters on histological examination nor elevated *H pylori* antibodies on EIA had a positive *CagA* value. This may be a false positive result because of cross reactive antibodies to *CagA*. In contrast, it has been suggested that *CagA* antibody measurement using immunoblotting may be more sensitive than EIA in showing past *H pylori* infection in some individuals.²⁹

In conclusion, atrophic gastritis was detected in 25% of consecutive outpatients referred for upper endoscopy. Moderate or severe atrophic corpus gastritis was found in 16 patients. Low serum pepsinogen I levels showed a sensitivity of 81% and specificity of 99% in detecting moderate or severe atrophic corpus gastritis, whereas high serum gastrin levels and the presence of parietal cell antibodies had high specificity (96%) but low sensitivity (75% and 56%, respectively). None of the blood tests reliably detected atrophic antral gastritis. *H pylori* and *CagA* antibodies were significantly associated with atrophic antral gastritis, whereas patients with severe atrophic corpus gastritis, most of whom had parietal cell antibodies, showed no signs of *H pylori* infection.

Our study was supported in part by Orion Diagnostica, Espoo, Finland, and the Yrjö Jahnsson Foundation, Helsinki, Finland.

We thank Mr Jarmo Ramberg for help in the measurement of serum gastrin. The skilful technical assistance of Ms Eila Kelo is gratefully acknowledged.

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