

Serum pepsinogen I and IgG antibody to *Campylobacter pylori* in non-specific abdominal pain in childhood

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SUMMARY A consecutive series of 51 children (mean age 11 years) who presented with recurrent abdominal pain were investigated by upper gastrointestinal endoscopy including three antral biopsies for microscopy, culture and urease testing. Serum IgG, IgA, and IgM antibodies to *Campylobacter pylori* (*C pylori*) were measured by the ELISA technique. Serum pepsinogen I was also measured. Thirty two children showed histological evidence of gastritis. All had *C pylori* on microscopy and or culture. Nineteen children showed no histological gastritis nor evidence of *C pylori* on microscopy, culture and/or urease testing. The IgG and IgA antibody levels to *C pylori* were significantly higher in *C pylori* positive children than in the negative group ($p < 0.001$). Serum pepsinogen I concentrations were also significantly higher in *C pylori* positive children than in negative ($p < 0.001$). Measurement of IgG antibody levels, combined with serum pepsinogen I estimation, predict the presence of *C pylori* associated gastritis in children with a sensitivity and specificity of up to 95%. It may be used therefore to predict gastritis and even peptic ulceration in children presenting with non-specific upper abdominal pain.

A strong association is now recognised in adults¹ between the presence of *Campylobacter pylori* (*C pylori*) in gastric mucosa and histologically confirmed gastritis. The question remains, however, whether *C pylori* is involved in the pathogenesis of acute or chronic gastritis, or is merely a commensal. *C pylori* also has been reported to be specifically associated with primary antral gastritis in children and as in adults, the organism is only present in the gastric mucosa in concert with gastric inflammation.² Raised serum pepsinogen I is found in about two-thirds of adult patients with peptic disease,³ and is thought to be a useful subclinical marker of genetic predisposition to ulceration.⁴ Serum pepsinogen I has recently been found to be raised in children with *C pylori* associated gastritis.⁵

We report the findings of a prospective study in which we used Giemsa staining, culture, and urease testing of antral biopsy specimens obtained from children undergoing upper gastrointestinal endoscopy as investigation of upper abdominal pain. We compared them with serum IgG, IgA, and IgM and serum pepsinogen I in order to assess the value of determination of these serological markers as a non-invasive guide to *C pylori* associated gastritis.

Methods

PATIENTS

Over a one year period, antral biopsies were obtained from 51 children undergoing upper gastrointestinal endoscopy for investigation of upper abdominal pain being evaluated from the presence of *C pylori*. Ages ranged from 1 to 18 years (mean 11). None of the children had received any medication which might affect gastric acidity before endoscopy.

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Fully informed parental consent was obtained before all endoscopies and biopsies.

ENDOSCOPY

Oesophagogastroduodenoscopy was performed under diazepam sedation using an Olympus GIF P3 gastroscope. The endoscope and biopsy forceps were disinfected in 2% glutaraldehyde after each use.

HISTOLOGY

Specimens were immediately fixed in 10% buffered formalin and stained with haematoxylin and eosin to determine the presence of gastritis, as described by Whitehead *et al.*⁶ Colonising *C pylori* was identified by Giemsa staining. The classification of gastritis was made without knowledge of the results of the Giemsa staining, culture, or urease testing.

CULTURE

The specimens were placed in 0.5 ml 20% sterile glucose. All were then processed within two hours and homogenised in 0.2 ml sterile water and then cultured on blood agar with amphotericin. They were incubated in a microaerobic environment at 37°C and examined after six days. *C pylori* colonies (small and grey) were tested for oxidase and urease and confirmed by Gram staining.

UREASE TEST

Before endoscopy, the CLOtest (Delta-West Ltd) was warmed to 30°C in an incubator or in the endoscopist's pocket as Marshall recommended.⁷ A 2 mm pinch biopsy was taken from antral mucosa and the tissue pushed beneath the surface of the CLOtest gels.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

C pylori isolated previously from one of our patients (B1) was used as the source of antigen. It was grown on blood agar, harvested, and sonicated for five minutes suspended in phosphate buffered saline. The sonicate was then centrifuged at 25000 g for 30 minutes and the supernatant used after a proper dilution as the antigen preparation in a standard ELISA technique. The antigen preparation was added to each well and incubated for two hours at 37°C. The plates were washed with washing buffer and binding sites were blocked by adding 2% serum albumin in washing buffer and incubated for 18 hours at 4°C. Diluted serum was added to each well and incubated for 90 minutes at 37°C. The plates were washed again and peroxidase linked IgG, IgA, and IgM conjugate was added and incubated for two hours at 4°C. The plates were washed again and substrate was added and left for 45 minutes in a dark

room and finally stopping solution was placed and the optical density of the wells was read at 470 nm using a MICROELISA plate reader.

SERUM PEPSINOGEN I

After an overnight fast, blood was drawn at 9 am and serum pepsinogen I concentrations were determined by radioimmunoassay (RIA) using a commercial kit (Pepsik, SORIN Biomedica, Saluggia, Italy). Results are given as ng/ml. The 99% confidence interval of serum pepsinogen I concentrations in a normal paediatric population have been previously studied* (43.2–56.7 ng/ml; mean=49.9, SD=9.8, 97th centile=69.5 ng/ml). A serum pepsinogen I level higher than 56.7 ng/ml was considered to be above normal.

STATISTICAL ANALYSIS

Statistical analysis by two tailed Mann-Whitney U-test was used.

Results

Table 1 shows the endoscopic findings in the children. Table 2 shows the results of histology, microscopy, culture, and urease testing in antral biopsy specimens obtained from 51 children. Thirty two of the specimens showed histological evidence of primary gastritis including all seven with chronic peptic ulceration. No children had secondary gastritis. The inflammatory infiltrate was generally mixed, consisting of plasma cells, lymphocytes and polymorphonuclear leucocytes. All 32 also had *C pylori* on microscopy. The remaining 19 antral specimens had normal histological features without evidence of gastritis or presence of *C pylori* on Giemsa staining, culture or urease testing. *C pylori* was cultured in 29 of

Table 1 Endoscopy results

Normal endoscopic findings	14
Oesophagitis	11
Nodular antritis	19
Gastric ulcer	1
Duodenal ulcer	6

Table 2 Results of microscopy, culture and urease test for *C pylori* in antral biopsy specimens from 51 children

	Subjects (n)	Presence of hist gastritis	Positive on Giemsa stain	Positive on culture	Positive urease test
Normal children	19	0	0	0	0
Children with primary gastritis	32	32	32	29	24

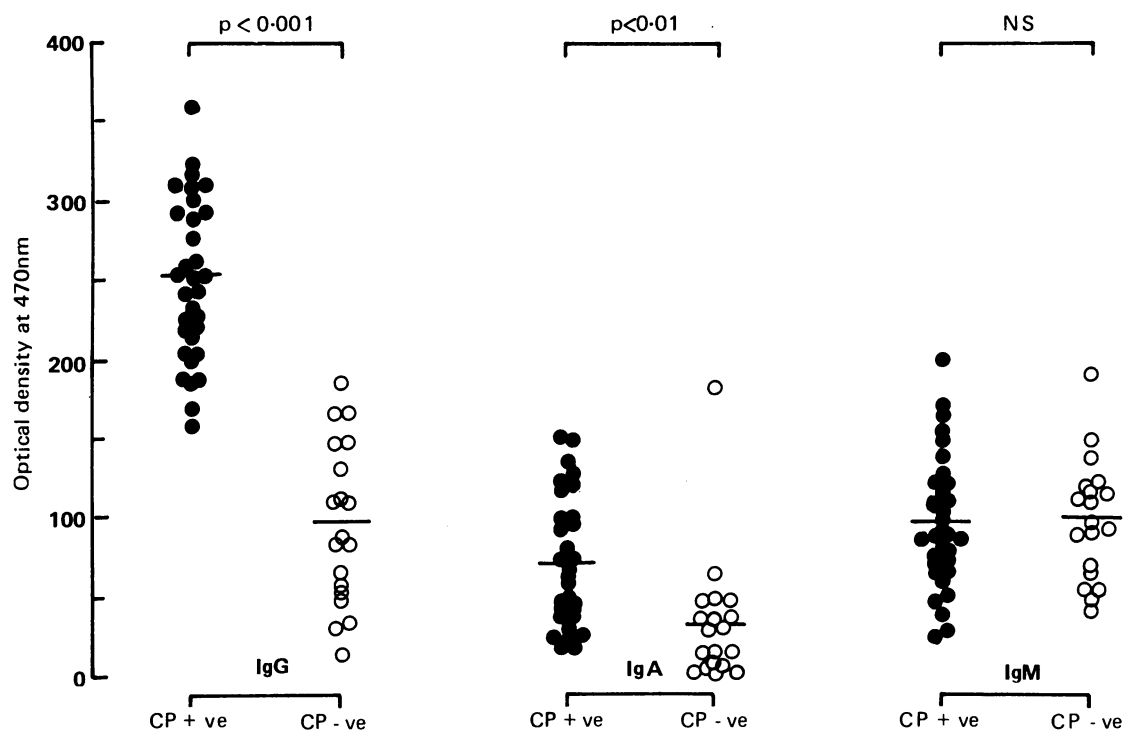


Fig. 1 Serum IgG, IgA, and IgM antibody titres to *C. pylori* in positive and negative children.

32 positive children (sensitivity 90%). The urease test showed positive results in 24 of 32 positive patients (sensitivity 74%). False positives were not observed with either technique (specificity 100%).

C. pylori positive children (*C. pylori* seen on microscopy and or culture) had significantly higher IgG and IgA than *C. pylori* negative patients ($p < 0.001$ and $p < 0.01$ respectively). IgM levels were not statistically different in *C. pylori* positive and negative groups (Fig. 1). Moreover, *C. pylori* positive children had significantly higher serum pepsinogen I concentrations than *C. pylori* negative patients ($p < 0.001$) (Fig. 2). Table 3 shows the sensitivity and the specificity of all the techniques used for the detection of *C. pylori*.

Discussion

Our results confirm that *C. pylori* is specifically associated with primary antral gastritis in children as in adults, the organism being found on the gastric mucosa only in the presence of antral inflammation as previously reported.² Further, significantly higher IgG ($p < 0.001$) and IgA ($p < 0.01$) levels were found in children with *C. pylori* associated gastritis, again mirroring adult investigations.⁹ The sensitivity and specificity of IgG testing was 93%.

Raised serum pepsinogen I is found in patients with peptic ulcer disease. It is inherited as an autosomal dominant trait,¹⁰ is correlated with gastric acid and pepsin secretion,¹¹ and may be a useful subclinical marker of genetic predisposition to peptic disease.⁴ Previous epidemiological studies have shown a weak but significant increase of pepsinogen I with age but this increase has only been found above the age of 30 years.^{12,13} We have previously calculated the normal range of serum pepsinogen I in children⁸ and that children with *C. pylori* associated gastritis have higher concentrations of serum pepsinogen I than children without *C. pylori* associated gastritis.⁵ Furthermore it has been shown that a normal serum pepsinogen I concentration in a child with peptic disease can predict that his disease will not relapse, while a raised concentration suggests an unfavour-

Table 3 Sensitivity and specificity of all test used for the detection of *C. pylori*

Microscopy	Urease		IgG	Pepsinogen I	
	Culture	test			
Sensitivity	-	90%	74%	93%	71%
Specificity	-	100%	100%	93%	71%

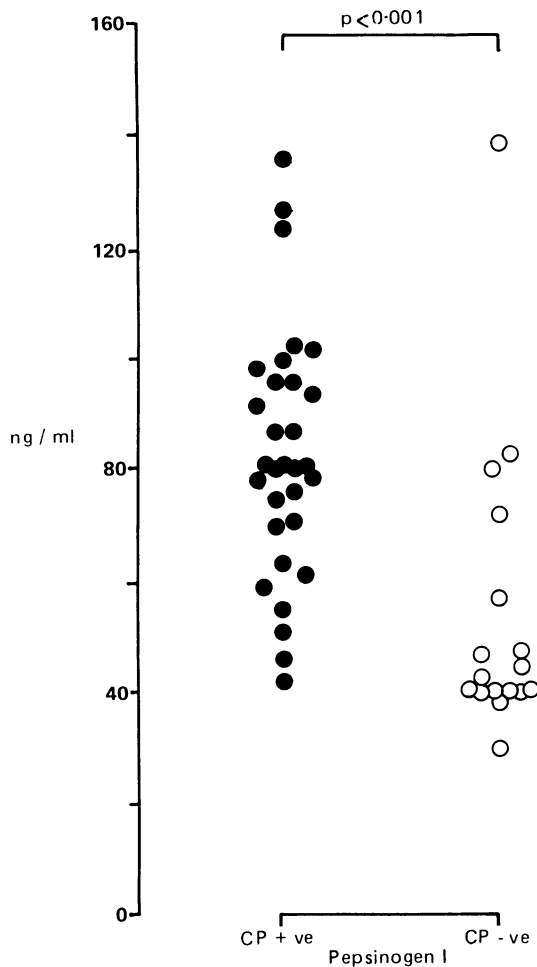


Fig. 2 Serum pepsinogen I in positive and negative children.

able outcome.¹⁴ In this paper we confirm the known rise of serum pepsinogen in children with peptic ulcer but have also shown that *C pylori* positive children with gastritis had significantly higher serum pepsinogen I than *C pylori* negative patients ($p < 0.001$). The sensitivity and specificity of a raised pepsinogen I for the presence of *C pylori* was 71%.

A combination of the two serological parameters (*C pylori* IgG; pepsinogen I) brings the sensitivity and specificity for *C pylori* detection up to 95%. They may be used therefore to predict the associated gastritis and/or ulceration and may be of value in deciding whether to endoscope a child with non-specific upper abdominal symptomatology. Moreover, as decrease in antibody levels with clearance of the bacteria and resolution of the histological

features of gastritis is known in adults,¹⁵ antibody levels may be helpful in following response to treatment of *C pylori* associated gastritis and peptic ulceration in children. Serology may also be used in population and longitudinal studies of the peptic ulcer diathesis.

Finally, it is tempting to speculate that as *C pylori* is associated with gastritis and peptic ulcer disease in children, inability to completely clear the organism after initial infection could conceivably result in a gastritis-ulceration progression with age. Raised pepsinogen I could then be involved as a primary or secondary phenomenon.

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