Helicobacter pylori may induce bile reflux: link between *H pylori* and bile induced injury to gastric epithelium

S D Ladas, J Katsogridakis, H Malamou, H Giannopoulou, M Kesse-Elia, S A Raptis

Abstract

***Helicobacter pylori* and duodenogastric reflux are both recognised as playing aetiological roles in chronic gastritis. This study investigated whether *H pylori* colonisation of the antral mucosa and duodenogastric reflux are independent phenomena or have a causal relationship. Thirty eight patients (15 men, 23 women) aged (mean (SD)) 48 (17) years participated. Each patient underwent gastroscopy. Antral biopsy specimens were taken to investigate *H pylori* colonisation. In addition BrIDA-^{99m}Tc/^{111}In-DTPA scintigraphy was used to quantify duodenogastric reflux. *H pylori* positive patients who were found to have duodenogastric reflux were treated with amoxycillin (1 g/d) and metronidazole (1·5 g/d) for seven days and four tablets of bismuth subcitrate daily for four weeks. Follow up antral biopsies and scintigraphy were repeated at six months. Duodenogastric reflux could not be found in 18 patients, including eight (44%) who were *H pylori* positive. Ten of the 11 patients who had duodenogastric reflux (reflux % 11·6 (9·2)), however, were *H pylori* positive (χ^2=6·26, p=0·01). These 10 patients were given eradication treatment. At six months, in six patients who became *H pylori* negative, duodenogastric reflux was significantly reduced from a pretreatment value of 14·3% to 3·3% (two tail, paired t=2·57, p=0·016). These data suggest that *H pylori* may induced duodenogastric reflux which may be important in the pathogenesis of *H pylori* gastritis or carcinogenesis, or both.**

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Keywords: *Helicobacter pylori*, bile reflux, duodenogastric reflux, *H pylori* gastritis, gastric carcinogenesis.

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Helicobacter pylori infection of the gastric mucosa is regarded as the major aetiological agent in chronic diffuse, superficial gastritis[1 2] and an important risk factor for the development of intestinal type gastric carcinoma,[3] though other factors, such as duodenogastric reflux (DGR) of bile, may play a part.[4] It has long been accepted that bile reflux affects the gastric mucosa[5 6] and is now regarded as an important cause of reactive gastritis.[4 7] However, published reports investigating a possible relationship between bile reflux and *H pylori* colonisation of the antral mucosa or *H pylori* gastritis, or both, have given conflicting results.[8–15] We therefore investigated whether *H pylori* colonisation of the antral mucosa and DGR of bile are independent phenomena or whether there is a causal relationship. This hypothesis was tested by measuring DGR before and after successful *H pylori* eradication in patients with intact stomachs.

Patients and methods

PATIENTS

Thirty eight patients, 15 men and 23 women aged mean (SD) 48 (17) years, participated in this prospective study. Ten patients had uncomplicated duodenal ulcer, two with second degree oesophagitis, and 26 had non-ulcer dyspepsia. No patient had had any abdominal surgery or known chronic liver disease. Women of child bearing age were not included in the study. The patients were informed about the nature of the study and all 38 agreed to participate. The protocol of the study was approved by the Ethical Committee on Human Studies, Department of Internal Medicine, University of Athens, in February 1992.

METHODS

Study design

On admission to the study each patient underwent upper gastrointestinal endoscopy and three antral mucosal biopsy specimens were taken from the lesser curve for a rapid urease test (CLO-test, Delta West, Bentley, Western Australia) and Gram stain biopsy smears. Patients in whom the results of the CLO-test and Gram staining were in agreement had ^{99m}Technetium-3-bromo-2,4,6-trimethylimino-diacetic acid/^{111}Indium-diethylenetriamine pentacetate (BrIDA-^{99m}Tc/^{111}In-DTPA) scintigraphy to quantify DGR. Those patients with both DGR and *H pylori* colonisation of the antral mucosa were treated with triple *H pylori* eradication therapy including 1 g amoxycillin and 1·5 g metronidazole daily for seven days and four tablets of colloidal bismuth subcitrate (De-Nol) daily, for four weeks. Similar triple therapy regimens are reported to have a *H pylori* eradication efficacy of between 72 and 90%.[16 17] At six months, all treated patients had follow up

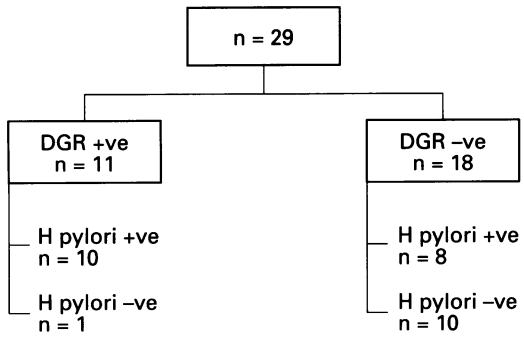

Figure 1: Of the 38 patients admitted to the study, nine were excluded because a reliable estimation of duodenogastric reflux (DGR) was not possible in two and in seven the CLO-test and Gram staining results did not agree. The 29 remaining patients had BrIDA-^{99m}Tc/^{111}In-DTPA scan to quantify DGR. Significantly more patients with DGR (DGR+ve) were H pylori *positive compared with patients without DGR ($\chi^2=6{\cdot}26$, df=1, $p=0{\cdot}012$).*

gastroscopy and antral biopsy specimens were taken to assess *H pylori* colonisation status. In those who became *H pylori* negative, BrIDA-^{99m}Tc/^{111}In-DTPA scintigraphy was repeated to quantify DGR.

Detection of H pylori

Both a rapid urease test and Gram staining were used to identify *H pylori* colonisation of the antral mucosa. One antral biopsy specimen was immersed in the yellow gel of the rapid urease test and gel colour change to pink (positive test) was checked for every 15 minutes over a two hour period. The CLO-test has a specificity of 97%, a sensitivity of about 95%, and gives a positive result at two hours in more than 80% of *H pylori* colonised patients.[18 19] Each of the remaining biopsy specimens (n=2) was crushed between two microscope slides, thereby producing four biopsy smears. All four smears were Gram stained and evaluated by an experienced bacteriologist (HM) for the presence and colonisation density of spiral bacteria, which appeared as red curved bacilli on Gram stain. The bacteriologist was 'blinded' to the results of the CLO test. Colonisation density was scored from one (<10) to four (>50 bacteria in at least one power field). *H pylori* positive patients were defined as those positive for both tests. Where the results of the tests did not agree, the patient was withdrawn from the study.

BrIDA-^{99m}Tc/^{111}In-DTPA scintigraphy

DGR was assessed by two senior staff (HG and MK-E) of the Nuclear Medicine Department, who were 'blinded' to the *H pylori* colonisation status of each patient.

After an overnight fast the patient received intravenously 2 mCi of BrIDA-^{99m}Tc, which is cleared selectively by the hepatocyte via the same pathway as bilirubin. Thirty minutes later, the patient was placed semirecumbent under a large field of view gammacamera (with a medium energy collimator) to obtain a baseline image. He then received a semi-liquid meal, consisting of two egg yolks beaten with 20 g of sugar, and drank 200 ml of water labelled with 100 μCi of ^{111}In-DTPA to delineate the gastric area of interest. Images of the abdomen were then acquired simultaneously in two windows (^{99m}Tc and ^{111}In) every five minutes for the next hour. The DGR index was calculated according to the formula $DGR=(S_t-S_o)/(H_o-H_t)\cdot 100$, where S_t and S_o are ^{99m}Tc-BrIDA activities over the stomach region at times t (max gastric activity) and zero (baseline image) respectively, whereas H_t and H_o are ^{99m}Tc-BrIDA activities over the hepatobiliary region at times t and zero respectively. The results were corrected for radioactive decay and blood background. BrIDA-^{99m}Tc/^{111}In-DTPA scintigraphy is the only non-invasive method available to quantitate DGR. The results of this method correlate highly with the actual concentration of bile acids recovered from the stomach.[20]

Statistical analysis

All data are presented as mean (SD). The statistical significance of the results was assessed by the χ^2 test with continuity correction factor or paired two tailed *t* test and regression analysis as appropriate.[21] A p value of less than 0·05 was regarded significant.

Results

Nine of the 38 patients admitted to the study (Fig 1) were excluded. In two of these patients superimposed jejunal loops on the gastric antrum did not allow a reliable estimate of DGR. In seven patients there was no agreement between the results of the CLO-test and Gram staining. Six of them had a positive CLO-test, but the Gram stain was negative. One additional patient had a negative CLO-test but the Gram stain was positive for *H pylori*-like bacteria. In the remaining 29 patients (Table), both the CLO-test and Gram stain were negative in 11 and positive in 18. Eleven patients were therefore defined as *H pylori* negative and 18 as *H pylori* positive.

In 18 of the 29 patients no DGR could be shown, but the remaining 11 patients had a mean (SD) DGR score of 11·6 (9·2)%. When combining the results of *H pylori* colonisation with those of DGR, 10 (91%) of the 11 patients who had DGR were found to be colonised by *H pylori*, but only 8 (44%) of those did not have DGR (n=18) were *H pylori* positive ($\chi^2=6{\cdot}26$, df=1, $p=0{\cdot}012$). There was no correlation between the DGR score and the density of *H pylori* colonisation ($r=0{\cdot}02$, $p=0{\cdot}7$), nor was the density of colonisation different in patients with (n=10) and without reflux (n=9) ($\chi^2=0{\cdot}3$, df=2, $p=0{\cdot}9$).

All 10 *H pylori* positive patients who had DGR underwent treatment to eradicate *H pylori*. One was lost to follow up at six months. Follow up endoscopy with biopsies showed that three of the nine patients were still colonised by *H pylori*, but six patients became *H pylori* negative (Table). After successful *H pylori* eradication treatment in the six patients, the DGR score was significantly reduced (Fig 2) from a pretreatment mean

TABLE I *Clinical data,* Helicobacter pylori *colonisation status of the gastric mucosa and results of duodenogastric reflux (DGR) measurements of the patients studied*

No	*Sex*	*Age (y)*	*Diagnosis*	H pylori *colonisation*	*1st DGR* (%)*	*Post eradication* H pylori *status*	*2nd DGR* (%)*
1	M	26	DU	+	3	−	2
2	M	27	OES	+	13	−	3
3	F	50	DU	+	22	−	5
4	F	63	NUD	+	10	−	0
5	M	30	DU	+	6	−	0
6	F	65	NUD	+	32	−	10
7	M	34	NUD	+	11	+	ND
8	F	41	NUD	+	4	+	ND
9	F	56	NUD	+	3	Lost	Lost
10	F	47	DU	+	12	+	ND
11	F	42	NUD	−	3	NFUP	NFUP
12	F	67	NUD	−	0	NFUP	NFUP
13	F	42	NUD	−	0	NFUP	NFUP
14	M	31	NUD	−	0	NFUP	NFUP
15	M	32	DU	−	0	NFUP	NFUP
16	F	60	NUD	−	0	NFUP	NFUP
17	F	43	NUD	−	0	NFUP	NFUP
18	F	68	NUD	−	0	NFUP	NFUP
19	F	43	NUD	−	0	NFUP	NFUP
20	F	62	NUD	−	0	NFUP	NFUP
21	M	58	DU	−	0	NFUP	NFUP
22	M	28	DU	+	0	NFUP	NFUP
23	M	72	OES	+	0	NFUP	NFUP
24	M	34	DU	+	0	NFUP	NFUP
25	F	73	NUD	+	0	NFUP	NFUP
26	M	69	DU	+	0	NFUP	NFUP
27	M	64	DU	+	0	NFUP	NFUP
28	F	40	NUD	+	0	NFUP	NFUP
29	M	35	NUD	+	0	NFUP	NFUP

DU=duodenal ulcer, NUD=non ulcer dyspepsia, OES=oesophagitis, ND=not done, NFUP=no follow up, Lost=lost to follow up.
*1st DGR=before treatment; 2nd DGR=after treatment.

value of 14·3 (10·9) to 3·3 (3·8)% (two tailed pair *t* test=2·57, p=0·016).

Discussion

It has long been accepted that the gastric mucosal barrier can be damaged by factors such as ingestion of aspirin and non-steroidal anti-inflammatory drugs, and by bile reflux. Bile acids and lysolecithin, were regarded as important factors in the pathogenesis of chronic gastritis and peptic ulcer.[22 23] During the past 10 years, however, these theories have been superseded by evidence indicating that chronic *H pylori* infection of the gastric mucosa is related to chronic gastritis[24 25] and duodenal ulcer[26 27] and is a risk factor for gastric carcinogenesis.[3]

Though *H pylori* and bile reflux gastritis are regarded as district histopathological entities, a number of studies have attempted to explain a possible relationship between *H pylori* gastritis and DGR of bile. Thus, it has been shown that bile is hostile to *H pylori* colonisation in vitro.[9 12 28] The effect of bile reflux on *H pylori* colonisation of the gastric mucosa has also been investigated in patients who have undergone gastric surgery. In one of these studies no significant difference could be shown in the quantity of bile reflux between *H pylori* positive and negative patients,[29] but two other studies came to the conclusion that postoperative bile reflux may play a role in the eradication of *H pylori*.[8 11] Furthermore, it has been shown that *H pylori* recolonises the mucosa of the gastric remnant after bile diversion (Roux-en-Y anastomosis).[30 31] Despite these observations, it seems that in the intact stomach bile reflux and *H pylori* often coexist[4 10] and both may therefore be involved in the pathogenesis of gastritis. This is because the bile acid concentrations of gastric aspirates sampled from patients who have not undergone surgery are usually much lower than those used in the in vitro studies[9 12 28] and those measured in postoperative stomachs.[30 31] The results of our study not only confirm that *H pylori* may survive the noxious effect of bile reflux in the intact stomach, but also that significantly more *H pylori* positive patients than *H pylori* negative patients may have DGR. We could not, however, show any significant correlation between the DGR score and the density of *H pylori* colonisation of the gastric mucosa, probably because of the patchy distribution of *H pylori* colonisation, which may result in tissue sampling error.

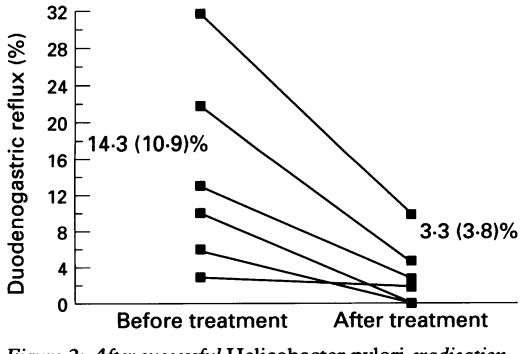

Figure 2: After successful Helicobacter pylori *eradication treatment duodenogastric reflux was significantly reduced in all six patients (two tailed paired* t=2·57, *p=0·016).*

The association between *H pylori* colonisation of the gastric mucosa and DGR was further investigated by comparing DGR before and six months after successful *H pylori* eradication. A long post treatment period was thought to be necessary for restoration of the gastric physiology after successful *H pylori* eradication. Though many investigators suggest that successful *H pylori* eradication is evident if *H pylori* can no longer be detected four weeks after the end of antimicrobial therapy, this length of time has been selected arbitrarily and most recurrences occur within six months, indicating that they may not be true recurrences but actually the result of incomplete *H pylori* eradication. Our observation that DGR is consistently reduced after successful *H pylori* eradication, implies that *H pylori* may actually be involved in the pathogenesis of DGR. This may be explained when considering that *H pylori* gastritis may alter gastric physiology by reducing the number of somatostatin producing cells of the gastric mucosa,[32] and by increasing both basal and meal stimulated gastrin release.[33] The increase of serum gastrin may affect antroduodenal motility and may be implicated in the pathophysiology of DGR.[34]

One limitation of this study is that we have investigated only postprandial DGR over a short period, while it is not known whether fasting or postprandial DGR is the more important and whether they coexist in the same patient. Certainly, a comparison of 24 hour DGR recording in *H pylori* positive and negative patients may provide us with more relevant information.

The importance of DGR as a cause of bile reflux induced gastritis and even of gastric

cancer is well recognised. Our data suggest that *H pylori* may induce DGR and therefore both may act synergistically on the gastric mucosa inducing chronic gastritis, which may lead to the carcinoma sequence. Indeed, it has recently been shown that the prevalence of intestinal metaplasia is greatest in those patients who have both *H pylori* associated gastritis and high intragastric bile acid concentration.[4]

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