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Cell proliferation in *Helicobacter pylori* associated gastritis and the effect of eradication therapy

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Abstract

***Helicobacter pylori* causes chronic (type B) gastritis. The 'intestinal' form of gastric cancer arises against a background of chronic gastritis, and prospective epidemiological studies have shown that *H pylori* is a major risk factor for this. An increase in mucosal cell proliferation increases the likelihood of a neoplastic clone of epithelial cells emerging where there is chronic epithelial cell injury associated with *H pylori* gastritis. In vitro bromodeoxyuridine labelling of endoscopic antral biopsy specimens was used to measure mucosal cell proliferation in *H pylori* associated gastritis before and after therapy for *H pylori* triple infection. Cell proliferation was increased in *H pylori* associated gastritis patients compared with normal controls and patients with *H pylori* negative chronic gastritis ($p=0{\cdot}0001$; Tukey's Studentised range). There was no difference in antral epithelial cell proliferation between duodenal ulcer and non-ulcer subjects infected with *H pylori* ($p=0{\cdot}62$; Student's *t* test). Antral mucosal cell proliferation fell four weeks after completing triple therapy, irrespective of whether or not *H pylori* had been eradicated ($p=0{\cdot}0001$). At retesting six to 18 months later (mean=12 months), however, those in whom *H pylori* had not been successfully eradicated showed increased mucosal proliferation compared with both *H pylori* negative subjects at a similar follow up interval and all cases (whether *H pylori* positive or negative) four weeks after completion of triple therapy ($p=0{\cdot}024$). These findings suggest that *H pylori* infection causes increased gastric cell proliferation and in this way may play a part in gastric carcinogenesis.**

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Keywords: Helicobacter pylori, type B gastritis, gastric cancer, cell proliferation.

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TABLE I *Antral mucosal cell proliferation in* Helicobacter pylori *associated gastritis*

	Group		
	Controls (n=12)	H pylori *−ve (n=10)*	H pylori *+ve (n=42)*
Mean (SEM) age (y)	42·3 (3·51)	34·4 (3·35)	43·8 (2·49)
Mean (SEM) LI%:			
Zone 1	0·7 (0·15)	1·0 (0·37)	1·3 (0·15)
Zone 2	11·5 (1·22)	10·3 (1·82)	17·9 (1·14)*
Zone 3	0·4 (0·15)	0·2 (0·09)	1·0 (0·16)
Total LI%	4·35 (0·51)	3·7 (0·65)	6·8 (0·28)*

LI=labelling index. One way analysis of variance indicates that both total antral gastric gland LI% and zone 2 LI% are increased in *H pylori* associated gastritis (+ve) compared with *H pylori* negative chronic gastritis (−ve) and normal control subjects ($p=0{\cdot}0001$; Tukey's studentised range). *Indicates that group is significantly different from the others.

The 'intestinal' form of gastric cancer, which is the commonest type, develops against a background of chronic gastritis, atrophy, and intestinal metaplasia.[1 2] *Helicobacter pylori* is the cause of (type B) chronic gastritis and has been shown in epidemiological studies to be a major risk factor for the development of gastric cancer.[3–6] Increased mucosal cell proliferation increases the likelihood of the development of a neoplastic clone of epithelial cells[7] where there is chronic epithelial injury associated with *H pylori* positive gastritis. However, little is known about cell proliferation in *H pylori* associated gastritis.

The aims of the study were, firstly, to compare antral mucosal cell proliferation in normal gastric mucosa with *H pylori* positive and negative chronic gastritis and, secondly, to determine the effect of *H pylori* eradication treatment on cell proliferation.

Methods

Patients undergoing routine diagnostic endoscopy were recruited after informed consent. Those taking non-steroidal anti-inflammatory drugs, H_2 antagonists, proton pump inhibitors, or bismuth salts, or those who had undergone gastric surgery, were excluded from the study. Using standard biopsy forceps, tissue specimens were taken from the gastric antrum (three) and corpus (two) for histological and immunohistochemical studies. The study was approved by the hospital ethical committee.

HISTOLOGY

Two antral and two corpus biopsy specimens from each site were routinely processed, and stained with haematoxylin and eosin.

H PYLORI

H pylori status was determined using a modified Giemsa stain on the antral and corpus sections and a biopsy urease test (CLO/DeltaWest) on the third antral biopsy specimen. To establish eradication of the micro-organism, both tests had to be negative.

IMMUNOHISTOCHEMISTRY

Two antral biopsy specimens for immunostaining were put immediately into RPMI (without L-Glutamine) (Gibco) containing bromodeoxyuridine (5 mg/10 ml). They were immersed in a waterbath for 60 minutes at 37°C then placed on filter paper and fixed in

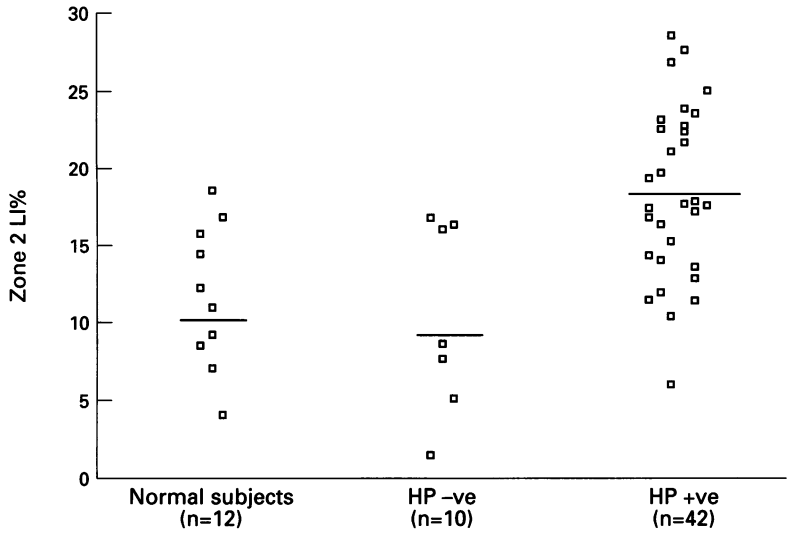

Figure 1: Antral zone 2 labelling index (LI%) is increased in Helicobacter pylori *chronic gastritis (HP+ve) compared with normal gastric mucosa and* H pylori *negative chronic gastritis (HP−ve) (p=0·0001).*

TABLE II *Antral mucosal cell proliferation in ulcer and non-ulcer associated* Helicobacter pylori *gastritis*

	Group	
	Non-ulcer gastritis (n=23)	*Ulcer gastritis (n=19)*
Mean (SEM) age (y)	45·6 (2·3)	41·9 (2·68)
Mean (SEM) LI%:		
Zone 1	1·1 (0·23)	1·5 (0·19)
Zone 2	17·6 (1·17)	18·3 (1·10)
Zone 3	0·6 (0·11)	1·5 (0·29)
Total LI%	6·7 (0·37)	6·8 (0·28)

LI=labelling index. No difference in total antral LI% and zone 2 LI% was observed between duodenal ulcer and non-ulcer associated *H pylori* gastritis (p=0·62; Student's *t* test).

formalin. Using a three step immunoperoxidase technique, sections were stained with anti-bromodeoxyuridine (DAKOPATT) antibody (1:20 dilution) for 60 minutes. Only sections that were complete and orientated were counted. For the purpose of counting, the gastric mucosa was divided into three zones: zone 1=surface and gastric pit; zone 2=isthmus; zone 3=glandular layer. The number of cells to be counted in each zone was determined by counting consecutive high power fields until the continuous mean varied by less than 5%. Five hundred cells were found to be necessary. The number of positively staining nuclei per 500 epithelial cell nuclei (or whole section when less than 500 cells per zone were present) was counted in each zone and expressed as a percentage. This value corresponds to the labelling index (LI%). The total LI% for the gastric glands was calculated from the sum of zones 1–3. Only unequivocally stained cells were counted as positive. All sections were counted by one person who was unaware of the subject's *H pylori* status.

ERADICATION THERAPY

Anti-helicobacter therapy consisted of a two week course of tetracycline 500 mg four times daily, metronidazole 400 mg three times daily, and tripotassium dicitrato-bismuthate 120 mg four times daily.

STATISTICAL ANALYSIS

Tukey's studentised range was used for three way analysis of data where appropriate. Student's *t* test was used for comparison of parametric data and Wilcoxon signed rank test for analysis of paired data. A p value of less than 0·05 was regarded as significant.

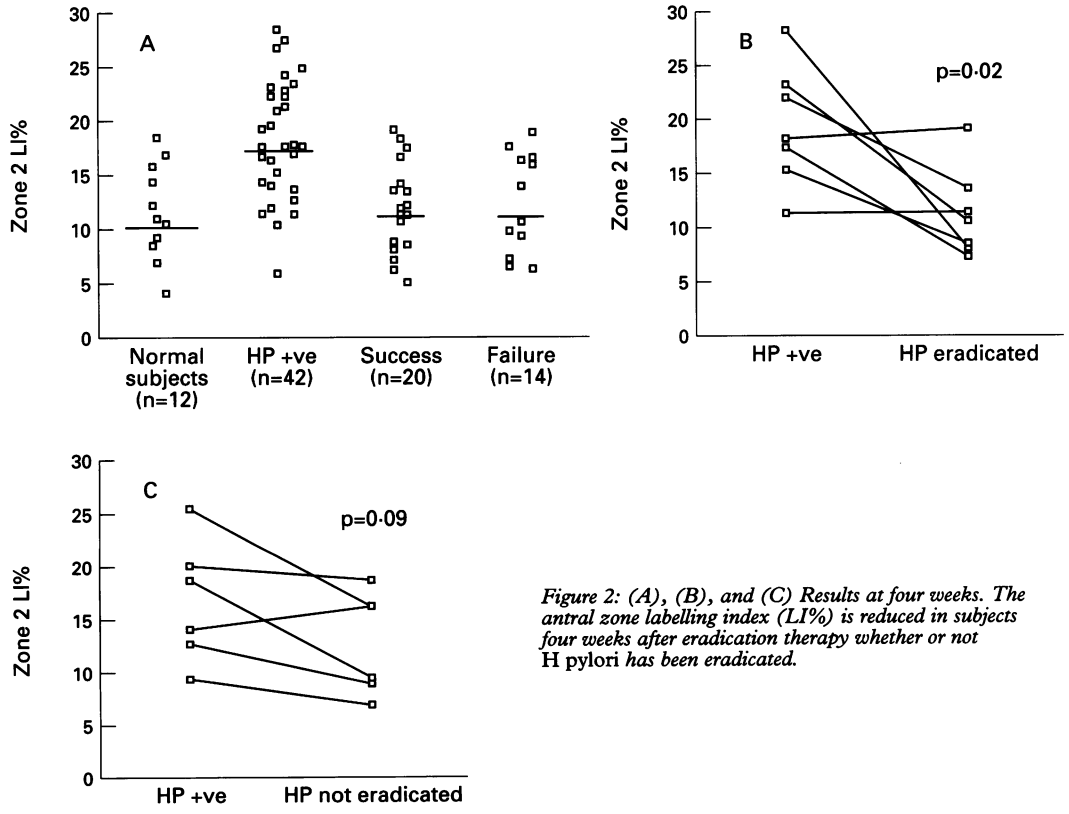

Figure 2: (A), (B), and (C) Results at four weeks. The antral zone labelling index (LI%) is reduced in subjects four weeks after eradication therapy whether or not H pylori *has been eradicated.*

TABLE III *Effect of treatment four weeks after completion on* Helicobacter pylori *positive patients*

	Group		
	H pylori +ve (n=42)	H pylori *eradicated* (n=20)	H pylori *not eradicated* (n=14)
Mean (SEM) age (y)	43·8 (2·49)	45·2 (2·65)	46·1 (3·02)
Mean (SEM) LI%			
Zone 1	1·3 (0·15)	0·9 (0·21)	1·4 (0·3)
Zone 2	17·9 (1·14)*	12·0 (0·96)	11·8 (1·22)
Zone 3	1·0 (0·16)	0·8 (0·2)	1·0 (0·26)
Total LI%	6·8 (0·28)*	4·5 (0·30)	4·9 (0·46)

LI=labelling index. Total antral LI% and zone 2 LI% are reduced in subjects four weeks after completing anti-helicobacter triple therapy whether *H pylori* has been eradicated or not (p=0·0001).

TABLE IV *Short term (four weeks) effect of anti-helicobacter therapy on antral cell proliferation*

	Group			
	H pylori *eradicated* (n=7)		H pylori *not eradicated* (n=6)	
Treatment status	*Total LI%*	*Zone 2 LI%*	*Total LI%*	*Zone 2 LI%*
Before	7·6 (0·86)	19·5 (2·1)	6·5 (0·81)	19·9 (2·37)
After	4·5 (0·53)	11·5 (1·48)	5·3 (0·71)	13·0 (1·96)
	p=0·02	p=0·02	p=0·03	p=0·09

LI=labelling index. Analysis of paired data from subjects before and four weeks after eradication therapy confirms the reduction in cell proliferation whether *H pylori* has been eradicated or not.

Results

CELL PROLIFERATION IN GASTRITIS (TABLES I AND II AND FIGURE 1)

Positive staining for bromodeoxyuridine varied in the same direction for all three zones. Most nuclei that stained positively for bromodeoxyuridine were situated in zone 2, corresponding to the isthmus or proliferative compartment of the gastric glands. Statistical comparisons between the different study groups are based on total LI% and zone 2 LI%. There was no difference in the LI% of normal control subjects and patients with *H pylori* negative chronic gastritis. However, both total and zone 2 LI% were increased in patients with *H pylori* associated chronic gastritis compared with normal control subjects and patients with *H pylori* negative chronic gastritis (p=0·0001). When the *H pylori* positive group was subdivided into those patients with duodenal ulcer and those with non-ulcer associated gastritis, no difference was detected in either the total or zone 2 LI%.

CELL PROLIFERATION AFTER ERADICATION TREATMENT (TABLES III–VI AND FIGURES 2–7)

Antral total and zone 2 LI% were reduced in patients four weeks after completing a course of anti-helicobacter therapy compared with untreated patients with *H pylori* associated gastritis (p=0·0001), whether the organism has been eradicated or not. This trend is confirmed by analysis of paired data from subjects before and after *H pylori* eradication therapy. Twelve patients underwent further biopsy a mean of 12 (range 6–18) months after completion of eradication therapy. Total and zone 2 LI% in eight *H pylori* negative subjects, that is, those treated successfully, were significantly lower (p=0·024) than those present in four *H pylori* positive patients. This trend is confirmed by analysis of paired data from subjects before and after *H pylori* eradication therapy.

Discussion

In this study we used the technique of in vitro bromodeoxyuridine labelling to identify cells in

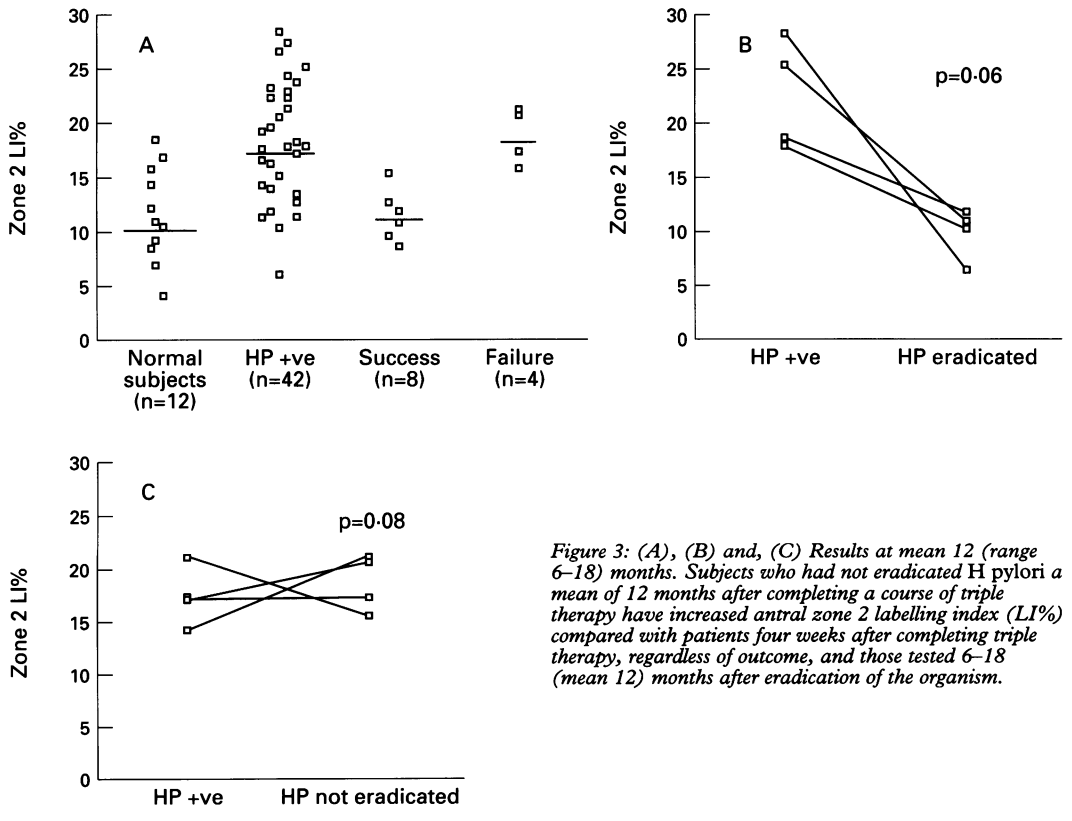

Figure 3: (A), (B) and, (C) Results at mean 12 (range 6–18) months. Subjects who had not eradicated H pylori *a mean of 12 months after completing a course of triple therapy have increased antral zone 2 labelling index (LI%) compared with patients four weeks after completing triple therapy, regardless of outcome, and those tested 6–18 (mean 12) months after eradication of the organism.*

TABLE V *Effect of time after eradication therapy*

	Group			
	4 Weeks after treatment		*6–18 Months after treatment*	
	H pylori *eradicated* (n=20)	H pylori *not eradicated* (n=14)	H pylori *eradicated* (n=8)	H pylori *not eradicated* (n=4)
Mean (SEM) age (y)	45·2 (2·65)	46·1 (3·02)	42·5 (5·14)	41·3 (7·04)
Mean (SEM) LI%:				
Zone 1	0·9 (0·21)	1·42 (0·3)	0·1 (0·08)	0·3 (0·09)
Zone 2	12·0 (0·96)	11·8 (1·22)	11·5 (0·98)	18·7 (1·29)*
Zone 3	0·8 (0·2)	1·0 (0·26)	0·2 (0·15)	0·2 (0·2)
Total LI%	4·5 (0·3)	4·9 (0·50)	3·7 (0·33)	6·4 (0·40)*

Subjects who had not eradicated *H pylori* a mean of 12 months after completing a course of triple therapy have increased total antral and zone 2 labelling index (LI%) compared with their values four weeks after completing triple therapy, regardless of outcome, and those tested 6–18 (mean 12) months after eradication of the organism (p=0·024).

the S phase of the cell cycle.[8 9] This method correlates well with the in vivo method[10 11] and was used in preference to the simpler technique of proliferating cell nuclear antigen (PCNA) immunostaining. PCNA is overexpressed in gastric mucosal biopsy specimens[12 13] and is a poor marker of cell proliferation at this site.[9]

We have shown that gastric mucosal cell proliferation, as measured by the labelling index, is increased in *H pylori* associated chronic gastritis but not in chronic gastritis where the organism is absent. This suggests that chronic inflammation alone is not associated with increased gastric epithelial cell turnover and that *H pylori* infection is required. Cytokines, free oxygen radicals, and other factors released by the inflammatory response after infection with *H pylori*[14 15] may interact with cells in the proliferative compartment to increase cell proliferation. The density of inflammatory cell infiltration in the region of the isthmus of the gastric glands is consistent with this explanation. Alternatively, the increased cell proliferation may be a direct compensatory response to accelerated cell loss, and be related to an increase in the activity of growth factors. Growth factor receptors are highly expressed in the proliferative zone.[16]

Previous studies have reported increased cell proliferation in gastric atrophy but not in superficial gastritis.[17 18] These studies did not specifically address *H pylori* status. Both *H pylori* positive subjects and those negative for the organism histologically may have been included in the studies. The combination of the two groups may prevent any statistical difference being detected when compared with subjects with normal gastric mucosa.

TABLE VI *Long term (mean 12 (range 6–18) months) effect of* Helicobacter pylori *eradication on antral cell proliferation*

	Group			
	H pylori *eradicated* (n=4)		H pylori *not eradicated* (n=4)	
Treatment status	*Total LI%*	*Zone 2 LI%*	*Total LI%*	*Zone 2 LI%*
Before	8·4 (0·99)	22·8 (2·5)	7·3 (0·58)	17·4 (1·37)
After	3·4 (0·41)	9·9 (1·2)	6·4 (0·4)	18·7 (1·29)
	p=0·06	p=0·06	p=0·5	p=0·8

Analysis of paired data shows that total antral and zone 2 labelling indices (LI%) are reduced towards normal a mean of 12 (range 6–18) months after eradication of *H pylori*, but remain increased where infection with the organism persists.

We have shown similar increases in antral mucosal cell proliferation in ulcer and non-ulcer patients. Non-ulcer associated gastritis usually affects both the antrum and corpus of the stomach (pangastritis) and is associated with a higher risk of gastric carcinoma than the predominantly antral, duodenal ulcer-associated gastritis. The difference in topographical distribution of non-ulcer associated gastritis is thought to account for the difference in cancer risk.[19 20] The histological and physiological changes that occur in pangastritis, namely atrophy and intestinal metaplasia in both antrum and corpus, lead to hypochlorhydria and proliferation of bacteria capable of nitrate reduction. This could result in production of nitrosocompounds which may be responsible for mutagenic events. The normal or increased acid levels in duodenal ulcer subjects would prevent this situation arising. The mucosal damage caused by *H pylori* may allow nitrosocompounds and other mutagenic agents such as bile and free radicals increased access to proliferating gastric epithelial cells.[2 21] Gastric juice vitamin C may play a role in the prevention of gastric cancer through its ability to scavenge oxygen free radicals and nitrite, a precursor of N-nitrosocompounds.[22 23] Vitamin C secretion is impaired in subjects with *H pylori* associated gastritis,[24 25] and eradication of the organism returns secretion to normal.[26]

We found that cell proliferation returned to normal four weeks after triple therapy, irrespective of the subject's *H pylori* status. This may have been a non-specific effect of treatment. Bismuth has anti-inflammatory effects[27] and may remain in the gastric mucosa for weeks after the completion of treatment. Because of this unexpected finding, patients were followed up with endoscopy and biopsy for a period ranging from six to 18 months after treatment. None of the patients had any other anti-helicobacter treatment in the interim. Those in whom *H pylori* eradication had been successful continued to have a normal mucosal proliferation rate but in those in whom eradication had failed cell proliferation returned to its previously high level.

This is the first report of the long term effect of *H pylori* eradication on gastric mucosal cell kinetics. Given that increased cell proliferation enhances the likelihood of genetic damage it seems reasonable to suggest that eradication of *H pylori* may reduce the risk of gastric neoplasia.[28]

In conclusion, we have shown that gastric mucosal cell proliferation is increased in *H pylori* associated gastritis and returns to normal after eradication therapy. This may have implications for gastric carcinogenesis and its prevention.

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