Parietal cells in the duodenal bulb and their relation to *Helicobacter pylori* infection

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Abstract

Aim—To investigate the prevalence, and relation to *Helicobacter pylori*, of parietal cells in the duodenal bulb using a monoclonal antibody directed against H⁺,K⁺-ATPase (HK12.18).

Methods—Twenty six patients with duodenal ulcer disease and 16 healthy controls were studied. *H pylori* status was determined by gastric histology and culture and by the ¹³C-urea breath test. Four biopsy specimens were taken from the duodenal bulb and stained with HK12.18. The presence/absence and number of parietal cells in the duodenal bulb were assessed blindly by a histopathologist.

Results—The overall prevalence of parietal cells in the duodenal bulb was 31% (13/42) and was similar in patients with duodenal ulcer and in controls, and in *H pylori* positive and negative subjects. The median (range) number of parietal cells in the duodenal bulb was 7.5 (4–20) parietal cells/subject, and was similar in all four groups.

Conclusions—The prevalence of parietal cells in the duodenal bulb (31%) is notably higher than previously reported in endoscopic studies, and is in keeping with reports from studies on necropsy/operative specimens. There was no difference in the prevalence or number of parietal cells in the duodenal bulb between patients with duodenal ulcer and controls, regardless of *H pylori* status. These findings suggest that parietal cells in the duodenal bulb do not contribute to the pathogenesis of duodenal ulcer.

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Keywords: *Helicobacter pylori*, duodenal ulcer, parietal cells, H⁺,K⁺-ATPase.

Parietal cells in the proximal duodenum were first described by Kaufman in 1906¹ and noted to be close to Brunner's glands. Further studies have shown their prevalence in the duodenal bulb to vary, ranging from less than 1%^{2,3} to almost 70%⁴ (table 1). Parietal cells were not found in the duodenum of the fetus and newborn, which suggests that they may be acquired.⁴ Most of these studies have described gastric heterotopia and not isolated groups of parietal cells in the duodenal bulb. Furthermore, there is some confusion in the literature regarding the difference between gastric heterotopia and gastric metaplasia in the duodenal bulb. The former refers to the presence of full thickness body-type gastric mucosa, and the latter, replacement of intestinal-type surface mucosa with gastric-type epithelium.

Widely reported variations in the prevalence of parietal cells in the duodenal bulb may be due to sampling error at endoscopy, or in the siting of the gastroduodenal junction.^{7,11} Detection of parietal cells may also be difficult by light microscopy on haematoxylin and eosin stained preparations. Complex trichrome and lectin stains have been used, but are time consuming and technically demanding.

The prevalence of parietal cells in the duodenal bulb has been reported to be higher in patients with duodenal ulcer disease.^{5,6,9} Johansen and Hansen⁶ found parietal cells in the proximal duodenum in 56 (22%) of 250 gastrectomy specimens (52 duodenal ulcer, 55 gastric ulcer, 23 prepyloric ulcer, and 120 cancer), but in 27 (52%) of 52 specimens taken from patients with duodenal ulcer (p<0.05). It has been suggested that production of acid by parietal cells in the duodenal bulb may contribute to the pathogenesis of duodenal ulcer.⁹ The relation between *Helicobacter pylori* infection, a major risk factor for duodenal ulcer, and parietal cells in the duodenal bulb has not been explored.

The aim of this study was to investigate the importance of duodenal parietal cells, rather than gastric heterotopia or gastric metaplasia, in the aetiology of duodenal ulcer. Prevalence of parietal cells in the duodenal bulb was determined in patients with duodenal ulcer and in healthy controls, with and without *H pylori* infection. Four carefully sited samples were taken and a highly specific and sensitive monoclonal antibody directed against H⁺,K⁺-ATPase (HK12.18) was used to identify parietal cells accurately.

Methods

Twenty six patients with endoscopically diagnosed duodenal ulcer were studied, 19 of

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Table 1 Prevalence of parietal cells in the duodenal bulb

Reference	Specimen type	Prevalence (%)
Taylor ²	Necropsy	2/150 (<1)
Leela <i>et al</i> ⁴	Necropsy	10/15 (67)
Hoedemaeker ⁵	Operative	
	All patients	52/158 (33)
	Patients with DU	27/52 (52)
Johansen <i>et al</i> ⁶	Operative	
	All patients	56/250 (22)
	Patients with DU	27/52 (52)
Tominaga ⁷	Necropsy	27/118 (23)
Shousha <i>et al</i> ⁸	Endoscopic biopsy	7/60 (12)
Wyatt <i>et al</i> ⁹	Endoscopic biopsy	5/518 (<1)
Carrick <i>et al</i> ⁹	Endoscopic biopsy	
	Overall	6/137 (4)
	Patients with DU	5/46 (11)
Koch <i>et al</i> ¹⁰	Endoscopic biopsy	69/337 (20)

DU = duodenal ulcer.

Table 2 Clinical and demographic details of the subjects studied

	Controls		Patients with DU	
Endoscopy	Normal	Normal	DU	DU
<i>H. pylori</i> status	Negative	Positive	Negative	Positive
No. of subjects	10	6	7	19
No. of men	4	5	6	13
Age in years mean (range)	33 (24–40)	34 (22–54)	39 (26–53)	37 (22–58)
Current smokers	3	2	1	3

DU = duodenal ulcer.

whom were positive for *H. pylori* infection. Six of the seven *H. pylori* negative patients had symptomatic and endoscopic recurrence of duodenal ulcer disease at least six months after eradication of *H. pylori*. The remaining *H. pylori* negative patient with duodenal ulcer had never been infected. Sixteen healthy controls (six *H. pylori* positive) with normal endoscopy were studied. None of the patients nor controls had taken non-steroidal anti-inflammatory drugs within one month, or antisecretory medication within two weeks of the study. Clinical and demographic details are given in table 2.

Figure 1 Duodenal bulb biopsy specimen stained with the monoclonal antibody directed against $H^+,K^+-ATPase$ (HK12.18). Labelled parietal cells are stained brown.

Figure 2 Gastric body biopsy specimen stained with the monoclonal antibody directed against $H^+,K^+-ATPase$ (HK12.18). Labelled parietal cells are stained brown.

DETERMINATION OF *H. PYLORI* STATUS

H. pylori status was determined by histology (antral, body and duodenal bulb biopsy specimens routinely processed and stained with haematoxylin and eosin and Gimenez stains), culture (antral and duodenal bulb biopsy specimens isolated in selective and non-selective media, and microaerobic conditions for up to 10 days) and by the ^{13}C -urea breath test (European Standard Protocol, where a result is positive if excess $\delta^{13}CO_2$ excretion exceeds 5 per ml¹²). Subjects were classified as *H. pylori* positive if they had a positive breath test and positive histology from any site or positive culture, or both. Absence of *H. pylori* was defined as failure to detect the bacterium in all three tests.

IDENTIFICATION OF PARIETAL CELLS

Murine monoclonal antibody HK12.18 was used as a specific marker for $H^+,K^+-ATPase$. HK 12.18 raised against pig gastric microsomes enriched in $H^+,K^+-ATPase$ was reconstituted from lyophilised mouse ascites with 250 μ l distilled water, aliquoted and stored at $-20^\circ C$ until required.

Four endoscopic duodenal bulb biopsy specimens were taken with standard cupped forceps (Olympus FB23-35K, Japan) by one endoscopist, more than 1 cm away from the edge of any duodenal ulcer, and more than 1 cm from the pylorus. The biopsy specimens were placed immediately in formalin. After routine processing, 2 μ m sections were stained with HK12.18 using a modification of the peroxidase-antiperoxidase technique.¹³ After washing with water and phosphate buffered saline (PBS), the slides were incubated with HK12.18 (diluted 1 in 2500) at $4^\circ C$ for 16 hours. They were then washed with PBS, and incubated for 30 minutes with peroxidase conjugated rabbit anti-mouse (dilution 1 in 300) sera (Sigma, Poole, Dorset, UK). Visualisation of the antigen ($H^+,K^+-ATPase$) was achieved by adding diaminobenzidine tetrahydrochloride hydrogen peroxide (DAB) as the chromogen substrate, and counterstaining with haematoxylin (fig 1). Figure 2 shows a gastric body biopsy specimen stained in the same way.

The presence or absence and the number of parietal cells in one section taken from each biopsy specimen was assessed blindly by an experienced histopathologist. The number of parietal cells is expressed as the total number of parietal cells seen in the four biopsy sections from each subject.

STATISTICAL METHODS AND ETHICS

The χ^2 test was used to compare the significance of differences between the groups, where $p < 0.05$ was regarded as statistically significant. The protocol was approved by Parkside ethical committee and all subjects gave written informed consent.

Results

The overall prevalence of parietal cells in the duodenal bulb in all subjects was 31% (13/42)

Table 3 Prevalence of parietal cells in the biopsy specimens of the duodenal bulb in healthy controls and in patients with duodenal ulcer (DU)

	Controls		Patients with DU	
No. of subjects	16		26	
Parietal cells present (%)	4 (25)		9 (35)	
<i>H pylori</i> status	Negative	Positive	Negative	Positive
No. of subjects	10	6	7	19
Parietal cells present (%)	3 (30)	1 (16)	4 (57)	5 (26)
Median (range) number of parietal cells/subject	6 (4-7)	20 (n=1)	7 (5-20)	7 (5-15)

(table 3). The prevalence of parietal cells in the duodenal bulb did not differ significantly ($\chi^2=0.66$, $p=0.4$) between patients with duodenal ulcer disease (nine of 26) and healthy controls (four of 16). The prevalence of parietal cells in the duodenal bulb was similar in all subjects, regardless of *H pylori* status (table 3). The median number of parietal cells in the duodenal bulb identified per subject was not significantly different between the four groups (table 3), with an overall median (range) of 7.5 (4-20) parietal cells/subject.

Discussion

IDENTIFICATION OF PARIETAL CELLS

Parietal cells, either isolated or in small clusters, may be difficult to identify using haematoxylin and eosin staining alone. We have used a highly specific and sensitive monoclonal antibody directed against H^+,K^+ -ATPase (HK12.18) to identify parietal cells within duodenal biopsy specimens; this technique has not been applied before. Smolka *et al*^{14,15} have confirmed the high specificity of HK12.18 in in vitro studies. The antibody has been shown to bind to the tubulovesicular membranes of the parietal cell cytoplasm.^{14,15} Kelly *et al*¹⁶ used a monoclonal antibody directed against H^+,K^+ -ATPase to map the distribution of parietal cells in the human fetal and infant stomach, and found that the site of monoclonal antibody binding corresponded precisely with the location of parietal cells as determined by staining with haematoxylin and eosin. Binding of a monoclonal antibody to vesicular membranes containing H^+,K^+ -ATPase was demonstrated by Karlsson *et al*.¹⁷ The same group showed that the monoclonal antibody directed against H^+,K^+ -ATPase did not bind to Na^+,K^+ -ATPase containing vesicular membranes, confirming its high specificity for H^+,K^+ -ATPase.

The high sensitivity of HK12.18 has been confirmed by Smolka *et al*,¹⁴ who demonstrated that the antibody bound to the tubulovesicular membranes of the parietal cell even after pre-treatment with cimetidine, so that antibody binding was independent of the secretory state of the cell.

PREVALENCE

The overall prevalence (31%) of parietal cells found in this study in the duodenal bulb is notably higher than in previous endoscopic studies, but in keeping with reports of necropsy and operative specimens (table 1). In the three largest studies of resected duodenal bulbs, parietal cells were found in 22-33% of cases.^{5,6,7}

By contrast, in the endoscopic studies parietal cells were found in only 1-20% of subjects.^{3,9,10} It has been reported that the prevalence of parietal cells in the duodenal bulb is higher in patients with duodenal ulcer than healthy subjects (table 1).^{5,6,9} Hoedemaeker⁵ found parietal cells in 52% of patients with duodenal ulcer compared with 33% of those without duodenal ulcer disease ($p<0.05$). Carrick *et al*,⁹ using two duodenal bulb biopsy specimens, found parietal cells more frequently in patients with duodenal ulcer than in healthy subjects, but the difference did not reach statistical significance. Using a highly specific method, we found that parietal cells were more common in the duodenal bulb in patients with duodenal ulcer than in healthy controls, but the difference was not statistically significant. According to our hypothesis, the difference in the prevalence of parietal cells in the duodenal bulb should have been largest between the *H pylori* positive patients with duodenal ulcer and the *H pylori* negative controls. However, the prevalence of parietal cells was similar between these two subgroups, with parietal cells seen in five (26%) of 19 *H pylori* positive patients with duodenal ulcer and in three (30%) of 10 *H pylori* negative controls. It is possible that our findings may be subject to a type II error—that is, the null hypothesis is not rejected when it is false. It would be necessary to study about 180 patients with duodenal ulcer and 180 controls to have a 90% power of achieving a significant result at the 5% level.

HISTOLOGY

Parietal cells were seen as single cells or in small clumps, and at most, in two of the four biopsy specimens from each subject (fig 1). The total number of parietal cells identified in the duodenal bulb biopsy specimens was small (median 7.5 per subject), and not significantly different between patients with duodenal ulcer and controls, or between *H pylori* positive and negative subjects. The sparsity of the parietal cells in the duodenal bulb may contribute to the difference in the reported prevalence between the necropsy and operative studies using larger pieces of tissue and the smaller endoscopic biopsy specimens. We suggest that taking four directed biopsy specimens from each duodenal bulb successfully minimised the sampling error.

A possible criticism of some of the earlier studies is that the parietal cells found in the duodenal bulb may actually have been at the gastroduodenal junction. This junction is usually sharp, and is characterised not only by the abrupt transition from gastric mucosa to the characteristic villous epithelium of the duodenal mucosa, but also by the transition from the branched tubular pyloric glands to multi-lobular mucus Brunner's glands.⁷ However, this junction is not always sharply demarcated and there may be islands of intestinal mucosa on the gastric side of the junction and, more rarely, islands of gastric mucosa on the duodenal side. The gastroduodenal junction may be crenated with finger-like processes of gastric

epithelium extending up to 6 mm into the duodenum.¹¹ We therefore took the duodenal bulb biopsy specimens more than 10 mm distally from the pylorus in order to avoid sampling gastric mucosa.

RELATION TO *H. PYLORI* INFECTION

Because *H. pylori* infection is a major risk factor for duodenal ulcer, and because the prevalence of parietal cells in the duodenal bulb was purported to be higher in patients with duodenal ulcer, we investigated whether there was a relation between *H. pylori* infection and parietal cells in the duodenal bulb in patients with duodenal ulcer. Basal and stimulated acid outputs are higher in *H. pylori* positive patients with duodenal ulcer than in *H. pylori* negative controls.¹⁸ It was therefore possible that *H. pylori* may have a similar effect on local acid production within the duodenal bulb. We hypothesised that *H. pylori* infection in patients with duodenal ulcer would lead to an increase in the prevalence of parietal cells in the duodenal bulb: it did not. In fact, the prevalence and number of parietal cells in the duodenal bulb was not affected by *H. pylori* infection, either in healthy controls or in patients with duodenal ulcer.

ROLE OF PARIETAL CELLS IN THE DUODENAL BULB

It is not known why parietal cells are found in the duodenal bulb. Carrick *et al.*⁹ demonstrated acid secretion within the duodenal bulb in patients with duodenal ulcer using Congo Red after stimulation with pentagastrin. Unfortunately, biopsy specimens were not taken from the apparent acid secreting areas (which turned Congo Red blue/black) to confirm that these sites did contain parietal cells, and that there was local acid secretion within the duodenal bulb. We had difficulty in replicating the Congo Red technique in patients with duodenal ulcer, because the often scarred and distorted duodenal bulb was not readily coated with the solution of indicator dye.

The parietal cells in the duodenal bulb identified with HK12.18 are probably functional, because they contain tubulovesicular membranes, which are an essential component of the acid secreting apparatus.^{14 15 19} However, we are unable to comment on the functional activity of these structures.

The number of parietal cells seen in the duodenal biopsy specimens was small, with a median of 7.5 cells seen per subject. Cox²⁰ estimated that the normal adult stomach contains about 1×10^9 parietal cells. An endoscopic biopsy specimen of the gastric body contains about 120 cells/mm². It seems likely therefore

that the amount of acid secreted by the small number of parietal cells in the duodenal bulb is negligible.

In conclusion, using multiple duodenal bulb biopsy specimens and a specific monoclonal antibody directed against H⁺,K⁺-ATPase to identify parietal cells, we have shown that these cells are found in the duodenal bulb in 31% of subjects. Their presence did not correlate with duodenal ulcer disease or *H. pylori* status. These findings do not support the hypothesis that parietal cells in the duodenal bulb contribute to the pathogenesis of duodenal ulcer.

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