**Helicobacter pylori** infection density and gastric inflammation in duodenal ulcer and non-ulcer subjects

S Khulusi, M A Mendall, P Patel, J Levy, S Badve, T C Northfield

---

**Abstract**

The factors that determine which *Helicobacter pylori* infected subjects develop duodenal ulcer (DU) are unclear. This study tested the hypothesis that infection density and urease activity are higher in DU than non-DU subjects. Fifty-five DU and 55 age and sex matched non-DU subjects were studied. Quantitative methods were used for measuring infection density (viable organism count) and urease activity (Berthelot reaction). DU subjects had a greater antral infection density (geometric mean of colony forming units/mg biopsy protein; 10^5 ± 3 x 10^5, p < 0.001). They also had higher biopsy urease activity (geometric mean of NH_3 nmol/min/mg protein; 103 ± 25, p < 0.001). Urease activity per organism, however, was similar in the two groups showing that high antral urease activity in DU was a reflection of organism density. DU was not present in subjects with an antral infection density less than 10^5 colony forming units/mg protein. A correlation was present between *H pylori* viable counts and the severity and activity of gastritis. Both severity and activity of gastritis were greater in the antrum of DU compared with non-DU subjects but there was no difference in the body between the two groups. It is concluded that antral *H pylori* infection density is probably an important determinant of DU development, and that there is a baseline of infection density that is necessary for ulcer formation.

(Gut 1995; 37: 319–324)

Keywords: *Helicobacter pylori*, infection density, gastric inflammation.

---

**Methods**

**Subjects**

Subjects were recruited from an open access endoscopy service. All were *H pylori* positive on the basis of serological tests performed prior to endoscopy as part of a separate *H pylori* screening study. Fifty-five DU patients and 55 age and sex matched subjects with no evidence of DU disease were recruited. Exclusion criteria were: acid suppressive treatment, anti-inflammatory drugs or antibiotics in the previous month; gastric ulcers, previous gastric surgery or duodenitis.
with no ulcer. Eight non-DU subjects were also excluded because no growth could be
detected on culture of their gastric biopsy
specimens and three who were *H. pylori*
negative on both histological examination and
culture. *H. pylori* was cultured from all DU
subjects recruited. Detailed patient information
including previous history of DU, family
history, smoking habit, and drug ingestion was
obtained by a research nurse using a
questionnaire before endoscopy. The DU and
non-DU groups were of similar ethnic origins;
smoking and a family history of peptic
ulceration were, however, significantly more
common in DU subjects (Table). The study
was approved by the St George’s Hospital
ethics committee and informed consent was
obtained from all patients.

**Infection density**

Three gastric antral and three body biopsy
specimens were obtained using the same size
forceps from similar topographical sites at each
endoscopy. Specimens were from the inferior
surface (2 and 3 cm away from the pylorus)
and from the superior surface of the antrum
(2 cm from the pylorus); and body biopsy
specimens from 5 cm above the angulus on the
lesser curve and two sites from the greater
curve opposite the angulus. The specimens
were covered with a drop of saline in individual
containers, maintained at 4°C, and processed
within two hours. Specimens were homo-
genised separately in 2 ml of normal saline, and
all samples were diluted to a protein
concentration of 50–100 μg/ml. Four aliquots of each
diluted homogenate were plated on Columbia
agar enriched with 5% lysed horse blood, and
incubated for five days. *H. pylori* colonies were
confirmed on the basis of morphology, Gram
stain, catalase test, and urease activity. They
were counted and the viable count expressed
as colony forming units (cfu) per mg of biopsy
protein.

**Biopsy urease activity**

Duplicate aliquots of homogenate were also
incubated with 50 mM urea at 37°C for one
hour. The ammonia produced was measured
by a photometric method using the Berthelot
reaction,\(^5\) giving a quantitative measurement
of biopsy urease activity, which was expressed
as nmol of ammonia per mg of protein per
minute.

**Gastric histology**

Two biopsy specimens from the antrum and
two from the body were obtained from each
patient. The specimens, from sites adjacent to
those used for viable count measurement,
were fixed in formal saline and embedded in
paraffin wax within 12 hours. Sections were
cut and stained with haematoxylin and eosin,
and assessed subjectively by one histopatholo-
gist for *H. pylori* density; sections were graded
0–3, corresponding to absent, scant, moderate, and heavy colonisation. Severity and activity of gastritis in the same specimens were also scored 0–3, corresponding to nil, mild, moderate, and severe mononuclear cell infiltration, and neutrophil infiltration respectively. The mean scores of the two specimens obtained from each area from the same subject were used, giving values on a seven point scale.

**Statistical methods**

Infection density in the antrum and in the body of each subject was expressed as the geometric mean of the viable counts from respective biopsy specimens. Urease activity was likewise expressed as the geometric mean of measurements from antral and from body biopsy specimens. Differences between means were compared by Student’s t test following log transformation of the data. Multiple regression was used to determine the relation between infection density and the conventional risk factors for DU. The relation between viable count and histological grade of infection density was assessed by Spearman’s rank correlation coefficient. This test was also used to assess the relation between viable counts and the histological parameters of inflammation.

**Results**

**Reproducibility**

To assess the effect of clumping at high infection densities, the reproducibility of viable counts at varying dilutions was assessed. Tenfold dilutions were performed on 20 of the specimens, and the counts were found to vary by 12%. The intra-assay coefficient of variation (CV) at the lowest concentration used was 10%. Intra-assay CV for the urease assay was 5%.

**Infection density**

Figure 1 shows that there was a close relation between infection density assessed by histological grading and that measured by viable count from the same area.

Figure 2 shows that mean *H pylori* density was more than seven times greater in the antrum of DU than non-DU subjects. Furthermore, there was a critical value of infection density (10⁵ cfu/mg) below which DU was not found. Infection density was significantly greater in the antrum than in the body of DU patients. In non-DU subjects, however, there was no difference in infection density between body and antrum. Gastric body infection density did not differ between the two groups.

Assessment of the conventional risk factors for DU showed that age, male sex, smoking, family history of DU, and ethnic group were not associated with infection density independently of DU.

**Urease activity**

Figure 3 shows that there was a significantly greater biopsy urease activity in the antrum of DU than non-DU subjects; and in the antrum compared with the body in DU subjects. As with infection density, however, there was no difference between either body and antral urease activity in non-DU subjects, or in the gastric body between DU and non-DU subjects.

Dividing biopsy urease activity by the viable count from the same biopsy we obtained the
urease activity/cfu, which was similar in the antrum and in the body of DU and non-DU subjects (Fig 4).

**Severity and activity of gastritis**

Figure 5(A) and (B) shows the correlation between viable count and the severity and activity of gastritis. Both severity and activity of gastritis were significantly greater in the antrum of DU compared with non-DU subjects (Mann-Whitney U test, p<0.001), but there was no significant difference in the body between the two groups (Fig 6).

**Discussion**

We have shown that both *H. pylori* infection density and biopsy urease activity are significantly greater in the antrum of DU subjects than those without DU. Urease activity per organism, however, is similar in these two groups showing that the higher antral biopsy urease activity is a reflection of greater organism density. Our data also suggest that there is a threshold level of infection density, which is necessary for ulcer formation.

The main limitation to enumerating *H. pylori* arises from the distribution of the organism at the mucosal surface. A high density of organisms results in a clumping effect, which causes more than one organism to contribute to the formation of a single colony when incubated on agar. Dilution of homogenates before incubation reduced this effect and further dilution made no additional difference, suggesting that at the concentrations used in this study clumping was not an important factor. A further limitation is definition of the two groups, as a number of subjects in the non-DU group may have a DU diathesis and will progress to ulceration in the future. These subjects cannot be considered as true controls, and could have biased this result against finding a greater difference between DU and non-DU subjects. Negative *H. pylori* cultures from eight non-DU patients probably resulted from low density of infection, and exclusion of results from these patients may have had a similar effect.

It is possible that an increased infection density in the antrum of DU subjects is an epiphenomenon arising as a result of an inflammatory or pathophysiological process associated with DU. In this study the severity
and activity of antral gastritis were greater in DU than non-DU patients. Antral inflammation could increase the availability of nutrients for _H. pylori_ as a result of higher gastric mucosal cell turnover, and so may contribute to an increase in the numbers of organisms. Gastric colonisation, however, almost undeniably precedes gastritis, making it less likely that inflammation determines infection density. There are a number of mechanisms whereby _H. pylori_ may produce gastritis. Its cytotoxin and urease have both been implicated in causing damage to the gastric mucosa both by toxic effects on mucosal cells and also by initiation of an ineffective chronic local immune response against the organism through the highly immunogenic nature of these proteins. Ammonia has been shown to have an additive effect to that of the cytotoxin on vacuolation of _HeLa_ cells in vitro showing that these two virulence factors have a synergistic effect, which may be important in vivo. A 128 kDa _H. pylori_ protein, associated with cytotoxin production has also been shown to produce a mucosal inflammatory response. It is possible that more severe inflammation results from greater and more damaging concentrations of ammonia, cytotoxin or 128 kDa protein in contact with the mucosa.

Physiological abnormalities of gastric acid secretion found in DU could determine the distribution of _H. pylori_. Acidity production in the body of the stomach may explain the greater numbers of organism in the non-acid producing antrum compared with the body, however, it is difficult to explain how the raised gastric acid secretion found in DU could favour a greater density of antral organisms compared with non-DU subjects. It seems more probable that _H. pylori_ itself is responsible for the increased acid secretion. McColl’s group have shown that sulcrate treatment, which in normal subjects has no inhibitory effects on acid secretion, produced a significant reduction in _H. pylori_ colonisation both on histological assessment and ^14^C urea breath test in DU subjects, and no change in corpus or antral gastritis scores. This was accompanied by a 50% reduction in the basal acid output, suggesting that infection density is an important determinant of the basal level of acid production. McColl’s group have also shown that gastrin releasing peptide stimulated a sixfold increase in gastrin and acid secretion in duodenal ulcer patients and only a threefold increase in _H. pylori_ infected non-DU subjects compared with uninfected controls. Differences in stimulated acid secretion between _H. pylori_ infected DU and non-DU subjects may also reflect the difference in antral infection density.

In this study we were unable to find any difference in the urease activity of strains from DU and non-DU subjects, which argues against there being ulcerogenic strains of _H. pylori_ with greater urease activity per organism. Other differences in _H. pylori_ strains, however, may be important in determining infection density. Expression of the 128 kDa protein, which is associated with DU but whose function remains unclear, may cause more severe gastritis by promoting greater _H. pylori_ colonisation.

_H. pylori_ density may be important in explaining how some infected subjects develop DU. We can speculate that a sufficiently high antral infection density and inflammatory response are needed to increase gastric acid secretion, an important factor in the development of gastric metaplasia in the duodenum. Colonisation of areas of metaplasia, which is more likely with a greater antral organism density then leads to chronic duodenitis, and this may further increase the extent of gastric metaplasia. Increasing duodenal colonisation and inflammation, in the presence of acid, ultimately results in mucosal disruption and ulceration.

The determinants of infection density may be important in explaining why some _H. pylori_ infected subjects develop complications such as DU. Apart from severity of inflammation we were unable to show any other convincing associations with infection density. More conventional risk factors for DU such as age, smoking, and male sex were not found to be independent determinants of infection density. Other factors, however, such as diet, blood group, and secretor status not considered in this study may influence the density of _H. pylori_ colonisation.

In conclusion this study has shown that DU is associated with increased _H. pylori_ infection density and suggests that a threshold of infection density in the gastric antrum is necessary for ulcer formation. Identification of the determinants of infection density may help to clarify the differences in outcome with _H. pylori_ infection.

Part of this study has been published in abstract form in _Gut_ 1993; 34 (suppl 4): S50.

SK was supported by the Astra Foundation Research Fellowship for Gastroenterology.


