Helicobacter pylori in Gambian children with chronic diarrhoea and malnutrition

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Abstract

Infection with *Helicobacter pylori* (formerly *Campylobacter pylori*) was studied by measuring antibody titres to *H pylori* in Gambian children. Serological evidence of infection was found in 12 of 82 (15%) infants aged less than 20 months; this increased to 62 of 135 (46%) in those aged 40–60 months. Positive serology was found in 41 of 77 (53%) infants with chronic diarrhoea and malnutrition (mean age 19 months, range 5–36) compared with 18 of 70 (26%) of age matched healthy controls and nearly a quarter (12/49, 24%) of age matched undernourished (marasmic) subjects. These data show that infection with *H pylori* is common in the Gambia and that in infancy this infection is associated with chronic diarrhoea and malnutrition.

Chronic diarrhoea and associated malnutrition is a major cause of childhood morbidity and mortality in developing countries. The pathogenesis of chronic diarrhoea and malnutrition is multifactorial,[1] and it is often associated with infection of the gastrointestinal tract. In a small percentage of affected infants pathogenic bacteria such as *Shigella* spp, *Salmonella* spp and enteropathogenic *Escherichia coli* are found.[2] More often micro-organisms of uncertain pathogenicity such as *Giardia lamblia* are identified.[3 4] Finally in most cases of well established chronic diarrhoea and malnutrition the small intestine contains high concentrations of colonic bacteria, which contribute to small intestinal dysfunction.[5 6]

The first major host defence against small bowel contamination, the gastric acid barrier,[7 8] may be compromised in children with protein energy malnutrition. Gracey *et al* described gastritis and achlorhydria in eight out of nine patients with protein energy malnutrition and, although the cause of the gastritis was not established, it was attributed, in part, to high bacterial counts found in the gastric contents.[9]

Infection with *Helicobacter pylori* (formerly *Campylobacter pylori*) seems a likely aetiological agent. This organism causes an active chronic gastritis and,[10] furthermore, it has become clear that *H pylori* gastritis may be associated with a rise of fasting gastric pH.[11–13] Diminished gastric acid output has persisted more than a year in several cases.[14] *H pylori* is found in adult populations around the world,[15 16] but is uncommon in children in the Western world.[17] The link between *H pylori* infection, achlorhydric gastritis, and bacterial overgrowth in the small intestine in children with chronic diarrhoea and malnutrition has not been investigated.

The aims of the study described in this paper were twofold: (1) to measure antibody titres to *H pylori* in Gambian patients with *H pylori* associated with gastritis, that had been proved histologically, so as to define antibody titres diagnostic of infection, and (2) to use serology to determine the prevalence of *H pylori* infection in Gambian children with chronic diarrhoea and malnutrition and in controls.

Subjects and methods

VALIDATION OF SEROLOGICAL METHOD OF DIAGNOSING CHRONIC INFECTION WITH *H PYLORI*

Fifty six subjects were examined for *H pylori* infection: 36 were adults referred because of a long history of dyspepsia (16 men, 20 women, mean age 34·7 years) and 20 were children. Of the children 17 had chronic diarrhoea and malnutrition, one had toddler diarrhoea, one (aged 9 years) had chronic dyspepsia, and one had recurrent respiratory infections and marasmus. Gastroscopy was performed on these subjects (method in infants as described previously[18]) and four biopsy specimens of antral mucosa were taken. Two specimens were put in sterile phosphate buffered saline for microbiological culture and two were fixed in buffered formalin solution for histological examination.

Specific anti-*H pylori* IgG was measured by enzyme linked immunoabsorbent assay (ELISA).[19] The antigen was prepared from whole organisms, using strains from the National Collection of Type Cultures (NCTC) (11637 and 11916), and strains of organisms isolated from individuals in The Gambia. Test sera were run on plates with a standard calibration serum derived from pooled *H pylori* positive sera from children in the United Kingdom. This serum was given an arbitrary ELISA value of 100 ELISA units. A calibration curve was constructed for each plate to allow each test serum to be given an ELISA value relative to the calibration serum. Each plate also had serial dilutions of a known negative control serum.

PREVALENCE OF ANTIBODIES TO *H PYLORI* IN A RURAL GAMBIAN POPULATION

Stored sera from 361 children aged less than 5 years resident in villages near the town of Farafenni on the north bank of The Gambia river were available for study. These sera were obtained during a malaria survey undertaken in November 1986 and represented each child

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Table 1 Details of children with chronic diarrhoea and malnutrition and two control groups: well and malnourished

Group	Age (months)		Sex (M/F)	No with marasmus/ marasmic-Kwashiorkor	Weight for height†	
	Mean (SD)	Range			Mean (SD)	Range
Chronic diarrhoea and malnutrition (n=77)	19·0 (6·3)	5–36	40/37	55/22	65·1 (7·9)	47–90
Well (n=70)	18·3 (8·6)	6–36	40/30	—	95·7 (3·7)	92–103
Malnourished* (n=49)	19·4 (7·7)	6–35	25/24	29/0	67·2 (4·9)	58–74

*These children had protein energy malnutrition.
†% NCHS median.

from every fifth compound in their respective villages. Basic demographic and anthropometric data were collected during the survey.

PREVALENCE OF ANTIBODIES TO *H PYLORI* IN CHILDREN WITH CHRONIC DIARRHOEA AND MALNUTRITION

Samples of sera were obtained from three groups of children: 77 with chronic diarrhoea and malnutrition; 70 asymptomatic well nourished children (weight for height >90% National Center for Health Statistics (NCHS) median value), and 49 malnourished children without diarrhoea (table 1). Chronic diarrhoea was defined as diarrhoea with more than three loose stools/day for more than two weeks and protein energy malnutrition when the weight for height fell below 75% of the NCHS median value. In patients with chronic diarrhoea and malnutrition the mean duration of diarrhoea was 12 weeks (range two to 52 weeks). The three groups were well matched for age and sex.

Marasmic control patients were recruited from the ward and outpatient department at the Medical Research Council (MRC) hospital in Fajara, and healthy controls were obtained with the help of field workers from the adjacent township of Bakau and from rural villages around Farafenni. Similar proportions of the groups of marasmic and healthy control children came from either a rural or an urban environment. *H pylori* infection was determined serologically and its prevalence in the three groups compared.

The protocol for the study was approved by the committee on human experimentation of the MRC Laboratories in The Gambia. Statistical analysis was performed by the χ^2 test and the *t* test where appropriate.

Results

ANTIBODIES TO *H PYLORI* IN PATIENTS UNDERGOING ENDOSCOPY

Antibodies to *H pylori* were measured in 36 adults and in 20 children who underwent gastrointestinal endoscopy. Satisfactory antral biopsies were obtained from 34 adult patients; 33 had antral gastritis. *H pylori* was identified in 32 patients by examination of Giemsa stained tissue sections (32/34) and Gram stained wet preparations (28/34). The organism was grown in pure culture from three patients. No bacteria were identified on the mucosa of the one patient with normal histology. Titres of specific anti-*H pylori* (NCTC) IgG were greater than 100 ELISA units in all adult patients in whom *H pylori* were identified. Similar results were obtained with antigens prepared from the NCTC strain of *H pylori* and the Gambian isolates. A similar association between raised antibody titres to *H pylori* and the demonstration of the bacterium on gastric mucosa was found in children. Sixteen of the 20 children endoscoped had antral gastritis. Eleven children had *H pylori* on their antral mucosa. Circulating IgG antibodies against *H pylori* were found in 13 children all of whom had antral gastritis (*H pylori* identified histologically in eight). They were not found in seven children: including all four with a histologically normal antral mucosa, two with atrophic gastritis but without *H pylori*, and in only one with gastritis and *H pylori* (table 2).

The titre of circulating antibodies exceeded 100 ELISA units in all adults and in eight out of the 13 children in whom *H pylori* was identified. In the remaining five infants the titre was more than 40 ELISA units dilution. Based on these findings in adults and children we concluded that an antibody titre of 100 ELISA units or greater strongly suggests an active infection with *H pylori*.

ANTIBODIES TO *H PYLORI* IN GAMBIAN VILLAGE CHILDREN

The prevalence of antibodies to *H pylori* at a titre of 100 ELISA units or greater in Gambian village children is shown in table 3. The prevalence of a raised antibody titre increased from 15% (12/82) children aged 0–19 months, to 27% (37/136) of those aged 20–39 months, and to 46% (62/135) in those children aged 40–60 months. Anthropometric data was available on these children and in those below 30 months of age there was a significant effect of malnutrition on the prevalence of high anti-*H pylori* antibody

Table 2 Correlation of anti-H pylori IgG with histological findings in gastric mucosa (n=20)

	H pylori present	H pylori absent
Antral gastritis (n=16)	9	7
Antibody titre (ELISA units):		
>100	8	—
10–100	—	5
Negative	1	2
No antral inflammation (n=4)*	2	2

*All four were negative on ELISA.

Table 3 Age related prevalence of anti-H pylori antibody titres

Age range (months)	Specific IgG titre >100 ELISA units (% prevalence)
0–19	12/82 (15)
20–39	37/136 (27)
40–60	62/135 (46)

titres. In this group 11 of 39 (28%) of those with marasmus (weight for height <75% NCHS median value) had antibody titres of 100 ELISA units or more compared with only 14 of 116 (12%) of those in the same age group who were well nourished ($\chi^2=4{\cdot}488$, 1 df, $p<0{\cdot}03$). This effect of malnutrition was not seen in the children over 30 months of age.

ANTIBODIES TO *H PYLORI* IN CHILDREN WITH CHRONIC DIARRHOEA AND MALNUTRITION AND CONTROLS

Antibodies to *H pylori* at a titre of 100 ELISA units or greater were found significantly more frequently in children with chronic diarrhoea and marasmus (41/77, 53%) than in children with maramus but no diarrhoea (12/49, 24%) or than in healthy age matched control children (18/70, 26%) ($\chi^2=9{\cdot}0$, 1 df, $p<0{\cdot}01$ and $\chi^2=10{\cdot}5$, 1 df, $p<0{\cdot}01$ respectively). Seventeen of the children with chronic diarrhoea and malnutrition also underwent endoscopy. *H pylori* was demonstrated on the mucosa of 10 of these children.

Discussion

In this study we have shown the value of the serological diagnosis of *H pylori* in the community under study and demonstrated that infection of the gastric mucosa with *H pylori* is established early in life in many children in The Gambia. The high anti-*H pylori* antibody titres found in The Gambia may reflect chronic infection and therefore prolonged antigenic stimulation. In a random population antibodies were found in 46% of children aged 40–60 months. In Britain the prevalence of infection in this age group is unknown but preliminary data from Newcastle suggests that it is around 1% and less than 6% for the age group 5–16 years.[17] The route of acquisition of *H pylori* infection is not yet established, but it is possible that person to person contact is involved.[20–22] Living conditions in The Gambia and especially feeding practices whereby children eat by hand from a communal bowl may favour the spread of *H pylori* and contribute to the high prevalence rate observed. Children in the tropics with chronic diarrhoea and associated malnutrition represent such a heterogenous group that obtaining adequate control groups for any study is difficult. This is illustrated by the apparent association between the prevalence of high anti-*H pylori* antibody titres and malnutrition in Farafenni children less than 30 months of age. This finding is, however, confounded by the fact that many of the malnourished children in this group also had concurrent diarrhoea. Therefore, an attempt was made in this study to control for the effects of both diarrhoea and malnutrition alone. Children with marasmus and no diarrhoea were not found to have a higher prevalence of *H pylori* infection than healthy children of the same age, whereas in the patients with marasmus in association with chronic diarrhoea, there was a significantly increased prevalence rate of *H pylori* infection compared with matched control groups. Chronic diarrhoea in association with marasmus may predispose to *H pylori* infection due to the fact that children with chronic diarrhoea and malnutrition are more unwell and more immunocompromised than children with marasmus alone. Alternatively, *H pylori* infection itself may predispose to enteritis with other organisms by compromising the gastric acid barrier. It is also possible that both factors operate. The association between gastritis associated with *H pylori* and hypochlorhydria is controversial.[23] Nevertheless, even a transient impairment of the gastric acid barrier at the time of infection could predipose to small bowel bacterial overgrowth and recurrent enteritis and may represent an important aspect of the pathogenesis of chronic diarrhoea and malnutrition in children in The Gambia. Further investigations of the relation between gastric *H pylori* infection, gastric acidity, and small bowel bacterial overgrowth in children with chronic diarrhoea and malnutrition are indicated.

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