Estimation of prevalence of Helicobacter pylori infection in an asymptomatic elderly population comparing [^{14}C] urea breath test and serology

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Abstract

A non-invasive serological assay devised in this laboratory had a sensitivity and specificity of 100% as determined by culture and confirmed by histology in a group of 47 patients who had undergone endoscopy. The correlation between serology and the non-invasive [^{14}C] breath test was very good. Only one of 24 culture positive patients was, while all 23 culture negative patients were, breath test negative. In a group of 46 healthy elderly persons, however, significant anomalies between serology and breath test were observed. Only 83% of the breath test negative persons were seronegative, while only 68% of the breath test positive persons were seropositive. These results can be explained in terms of age related atrophic gastritis and immune incompetence, causing reduced colonisation and decreased antibody production, respectively.

These investigations suggest that non-invasive tests for *H pylori* infection may not be reliable in the elderly.

Seroepidemiological studies have shown that there is an age related rise in the prevalence of *Helicobacter pylori* infection in normal populations, such that in the United States of America and United Kingdom[1] the rate of acquisition of infection is about 1% a year. This age related increase in *H pylori* infection has also been confirmed using the urea breath test[2] and correlates with a previously established age related increase in histologically confirmed gastritis.[3] In several seroepidemiological studies, however, this rate of increase slows and frequently falls in the older age groups.[4] Nevertheless, such an effect was not observed in epidemological studies using the [^{13}C] urea breath test.[2] Whether this decrease reflects an absence of *H pylori* infection or merely a deficiency in the serological assays is not known.

The aim of this study was to determine the prevalence of active *H pylori* infection in a healthy group of elderly subjects using a [^{14}C] urea breath test. The presence or absence of *H pylori* in this population, as determined by the breath test, was then related to seropositivity. The sensitivity and specificity of both the breath test and the serological assay, against *H pylori* culture positivity, were established in a group of patients who had undergone endoscopy.

Methods

Two groups of patients were investigated. Group 1 comprised 47 patients, aged 20–79 (mean 41 years) referred for gastroscopy on the basis of clinical symptoms. At endoscopy, biopsy specimens from three sites were taken for microbiological and histological examination. On the morning of the test the patients underwent a [^{14}C]-urea breath test and gave a blood sample for serology.

Group 2 comprised 46 elderly asymptomatic patients (aged 65–84 years; mean 73 years) selected from the age/sex register of two general practitioners. Volunteers were sought who were considered fit and healthy by the general practitioner. Persons who had a history of upper gastrointestinal tract disease within the previous 15 years, or those who had any serious illness with the past year were excluded. Those receiving regular medication for indigestion, or treatment known to disrupt the gastric mucosa such as non-steroidal anti-inflammatory drugs (NSAID), or those who had received antibiotics over the preceding fortnight were excluded. Informed consent was obtained and each patient was given a [^{14}C] urea breath test and a blood sample was taken for serology on the same day. All patients fasted overnight and were weighed before the [^{14}C] urea breath test. Groups 1 and 2 were given 370 kBq and 185 kBq [^{14}C] urea (Amersham), respectively, in 100 ml of distilled water. Exhaled breath was collected as previously described.[5] Breath was sampled at five, 10, 15, 20, 30, 40, 50, 60 and 120 minutes, or five and 15 minutes for groups 1 and 2, respectively. Toluene based scintillant was added and the ^{14}C activity measured by liquid scintillation (Kantron Betamatic liquid scintillation counter). The activity was expressed as the percentage adminstered dose (AD)/mmol carbon dioxide × body weight (kg). Using this procedure in group 1 the peak percentage AD/mmol carbon dioxide × body weight was found to occur at five, or 15 minutes, or both.

Biopsy specimens were cultured for *H pylori* as previously described.[6] Growth of *H pylori* was confirmed by the characteristic morphology and urease production. For histological identification of infection, glutaraldehyde fixed (5% glutaraldehyde in 0·1M cacodylate buffer, pH 7·3) biopsy specimens were embedded in resin and 1·0 μm section cut as described previously.[6]

Sera from all patients were assayed for *H pylori* IgG antibodies using the acid extractable surface antigens from *H pylori* NCTC

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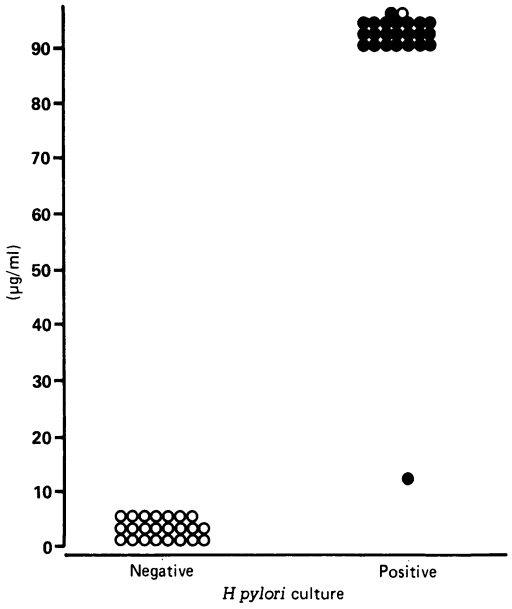

Figure 1 Concentration of H pylori IgG antibodies in the serum of H pylori culture positive and negative patients who were [^{14}C] urea breath test positive (●) (patients with a % AD/mmol carbon dioxide × body weight of over 0·5) or negative (○).

11638 in an ELISA as previously described,[7] except that the dilution of human sera was 1 in 200. The concentration of specific antibody was derived from a standard curve of IgG mass against optical density at 350 nm as described previously.[7]

Results

The positivity of the [^{14}C] urea breath test and the serological test was established in the group of endoscoped patients. Twenty three of 47 (49%) were infected with *H pylori* as determined by culture and confirmed by histological observation. The correlations between the [^{14}C] urea breath test and serological test in *H pylori* positive and negative patients are given in fig 1. On the basis of this information a peak percentage AD/mmol carbon dioxide × body weight of 0·5 was taken as the threshold for breath test positivity. This peak occurred between five and 15 minutes after the administration of the [^{14}C] urea. All 23 (100%) of the culture negative patients were breath test negative but only 23 of the 24 (96%) of the culture positive patients were breath test positive. The threshold for seropositivity was taken as 10 μg/ml. All culture positive patients (100%) were seropositive and all culture negative patients (100%) were seronegative.

To cause as little inconvenience to the elderly patients as possible the breath test was slightly modified so that only two breath samples were taken at five and 15 minutes. The peak percentage AD/mmol carbon dioxide × body weight was calculated at these times after administration of the [^{14}C] urea. This modification did not affect the sensitivity or specificity of the breath test. The relation between breath test and serology in the elderly patients is given in fig 2. Using the threshold established previously, 18 of 46 (39%) elderly persons were breath test negative; 15 (83%) of these were seronegative. Of the 28 who were breath test positive, only 19 (68%) were seropositive.

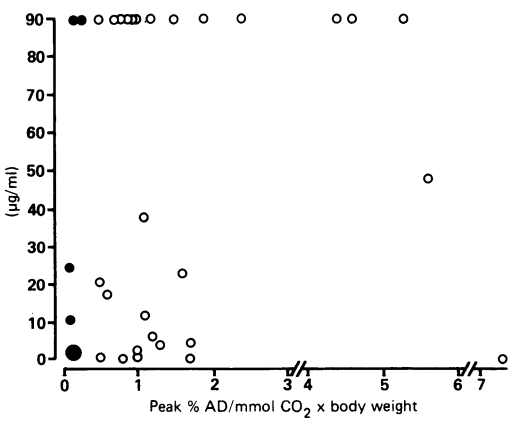

Figure 2 Concentration of H pylori IgG antibodies relative to [^{14}C] breath test positivity (○) or negativity (●) in elderly patients. Fourteen sera had antibody titre lower than 6 μg/ml and a % AD/mmol carbon dioxide × body weight of under 0·5.

Conclusions

Both the [^{14}C] urea breath test and the serodiagnostic test are indirect measures of *H pylori* infection. Their major advantage over the direct biopsy based techniques of diagnosis, however, is that they are non-invasive, which allows epidemiological studies in normal populations to be performed. The sensitivity and specificity of both tests used in this study has been established with endoscoped patients whose positivity or negativity for *H pylori* is known, determined by biopsy specimen culture and confirmed by histological detection. The performance of both tests was similar to that observed previously,[5 7] confirming that the [^{14}C] urea breath test and serological test were excellent predictors of *H pylori* infection in patients who had undergone endoscopy and, presumably, the general population. The results obtained with the asymptomatic elderly population, however, were more difficult to interpret. Of the breath test negative persons, three of 18 (16%) were serologically positive. There are several possible explanations for this anomaly. As low numbers of organisms could significantly reduce the sensitivity of the breath test these three patients may have had low grade infections. These patients might also recently have overcome their infection, the [^{14}C] urea breath test being an indicator of current infection. Conversely, circulating specific IgG antibodies are known to remain high after successful eradication in some patients.[8] There is no evidence to suggest that *H pylori* infection can be eradicated without extensive therapeutic intervention.[9] Nevertheless, increased prevalence of atrophic gastritis occurs with age[10] and such an environmental condition seems to be incompatible with *H pylori* colonisation and could, therefore, account for significantly decreased numbers of infective organisms in the elderly.

Of more concern to the value of the serodiagnostic test is the high proportion of elderly subjects (nine of 28; 32%) who were breath test positive but seronegative. Seronegativity is recommended as a basis for the deselection of patients from endoscopy procedures.[11] Eight of nine of these seronegative persons had sig-

nificantly positive breath tests, indicating that *H pylori* was present at the gastric mucosa. The absence of serum antibodies in *H pylori* positive patients is not unknown. This is not a reflection of antigenic variation between *H pylori* strains but rather the immune incompetence of the patients.[12] Such patients, however, usually represent substantially less than 10% of the *H pylori* positive population.[7] This proportion seems to increase significantly in the elderly population. It is strongly recommended that patients over 45 years of age undergo endoscopy regardless of *H pylori* serological state to include those at higher risk from gastric cancer.[13] These serological results on the elderly reinforce this recommendation. The reasons for the absence of *H pylori* antibodies in these patients are under investigation. Mucosal surface immune incompetence of the elderly is well documented[14] and is probably a function of reduced T cell populations as a result of thymic tissue loss which will cause a decline in antibody responses to T cell dependent bacterial antigens.

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