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Use of serology, the urease test & histology in diagnosis of *Helicobacter pylori* infection in symptomatic & asymptomatic Indians

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Age-specific prevalence of IgA and IgG antibodies in 714 subjects without gastrointestinal complaints aged 6 months to 90 yr was measured by an enzyme linked immunoassay using an acid-glycine extract of *H. pylori* as the antigen. The urease test and histology were used for the diagnosis of *H. pylori* infection in 83 subjects with a clinical diagnosis of dyspepsia, and these results were compared with measurement of IgG, IgA and IgM antibodies. The age specific prevalence of IgG and IgA antibodies respectively was 57 and 43 per cent for subjects aged 6 months to 4 yr and showed an increase with age to a maximum of 90 per cent for IgG in subjects >60 yr of age and to 87 per cent for IgA in subjects between 51 and 60 yr. In symptomatic patients, there was a high degree of correlation between severity of *H. pylori* infection on histopathological examination and IgG ($P<0.02$) levels. The use of IgG and IgA estimation could have identified *H. pylori* infection without endoscopy in 50 of the 83 patients. Serology for IgG and IgA antibodies against *H. pylori* may play a major role in decreasing the need for endoscopy, but cut-off values must be determined for each assay based on the prevalence of antibodies in the population.

Key words Epidemiology - *Helicobacter pylori* - histopathology - Indians

Helicobacter pylori is strongly associated with a spectrum of disease that ranges from nonulcer dyspepsia and peptic ulcer disease to gastric tumours[1]. This spiral organism causes a chronic bacterial infection, appropriate treatment of which results in eradication of the organism, histological evidence of healing and a reduced relapse rate[2]. Diagnosis of infection due to *H. pylori* can be made by histology, the urease test and culture, all of which require endoscopic biopsy, an invasive procedure[3], and by non-invasive techniques such as the urea breath test[4] and serology[5-7]. Among the non-invasive techniques, the urea breath test which uses carbon isotopes requires the use of a scintillation counter or mass spectroscopy. Serology has the advantages of being fast, inexpensive, non-invasive and requiring an ELISA reader which is available in most diagnostic laboratories. It has been estimated that serological diagnosis of *H. pylori* infection is capable of reducing the endoscopy work load by 23 per cent[8]. However, published studies on the epidemiology of *H. pylori* infection show considerable differences in the prevalence of antibodies to *H. pylori* in different populations[9,10].

In India, studies have shown that by the age of 20 yr, over 80 per cent of the population acquires antibodies to *H. pylori*[10,11], although one study that examined sera from blood donors found antibodies in only

54 per cent[12], indicating that data on antibody levels in asymptomatic and symptomatic populations are required before serological tests can be used to evaluate the presence of infection and possibly the degree of colonization.

This study was carried out to determine the seroprevalence of antibodies in patients, presenting to hospital without gastrointestinal symptoms, and use the data obtained to determine the correlation between IgG, IgA and IgM antibody titres and density of *H. pylori* colonization and severity of gastritis on biopsies from Indian patients undergoing endoscopy with clinical, pre-endoscopic diagnoses of dyspepsia.

Material & Methods

Subject selection and sampling procedures : For the first part of the study to determine age-specific prevalence of antibodies to *H. pylori*, 714 serum samples were selected from sera sent to the Clinical Biochemistry Laboratory from all services of the hospital excluding gastroenterology. Data on age and sex and registration number were available for all samples to ensure that duplicate samples from the same patients were not processed. All samples from children less than 6 months of age were excluded.

For the second part of the study to compare the urease test, histology and serology, all patients (n=83, age range 19-66 yr) undergoing endoscopy for dyspepsia between June and September 1994 were included in the study. For each patient, the following procedures were performed: clinical evaluation, upper gastrointestinal endoscopy, gastric biopsy (antrum and corpus) and serum collection.

Urease test : One piece of the biopsied tissue was immediately put into 10 per cent urea broth (Difco, Detroit, USA) containing a phenol red indicator. The broth was examined every 15 min for up to 2 h and the time taken for the indicator to turn pink was noted.

ELISA : The antigen preparation and ELISA procedure were as reported previously[13]. The antigen was an acid-glycine extract of *H. pylori* (a gift from Professor T. Wadstrom, University of Lund, Sweden). The sera were tested in duplicate and appropriately diluted positive and negative sera were included in each plate. Optical density values at 405 nm were measured in a Biorad (USA) ELISA reader. In order to determine the cut-off values of optical density (OD) in the assay, 10 cord blood sera were collected and tested. The 5 sera with the lowest values were tested three times in triplicate to establish intra- and inter-assay variation which was found to be less than 10 per cent. The two positive sera included in each assay were obtained from patients with acid peptic disease with histological evidence of gastritis and *H. pylori* infection, these had previously been tested in a commercial ELISA (Helico G, Porton, Cambridge, UK). The mean and 2 standard deviations (SD) were used to establish cut-off values for IgG (0.200) and IgA (0.160). The cord sera were negative for IgM.

Histopathology of gastric biopsies : Gastric biopsies were collected in 4 per cent formalin, processed for routine histopathological examination, and stained with haematoxylin and eosin, and with modified Giemsa stain. These were assessed for evidence of acute or chronic gastritis, degree of inflammation, atrophy, metaplasia and infection with *H. pylori* and scored on a scale of 0 to 3, for no changes and mild, moderate or severe changes.

Data analyses : The asymptomatic subjects were divided into 8 age groups. The arithmetic mean of the optical densities for each age group were calculated. Overall means and standard deviation were determined. Correlation and two-tailed *P*-value were obtained for IgG and IgA. For the group of symptomatic patients, positive histology and/or a positive urease test were used as the gold standard and sensitivity and specificity were calculated for ODs for each class of antibodies.

Results

The age and sex distribution of asymptomatic study population is shown in Table I. The minimum age was 6 months and the maximum age was 90 yr. The male-female ratio was 1.95:1. Of a total of 714 subjects, 576 (80.0%) and 556 (77.8%) had IgG and IgA antibodies to *H. pylori*, respectively. The frequency of both antibodies increased with age (Table I). The youngest age group had the least prevalence of both IgG and IgA antibodies, but there was a marked increase in the prevalence of both antibodies up to the age of 19 yr, reaching 80 per cent prevalence levels. The age group 30 to 39 yr had a slight decrease in percentage of subjects

with IgG antibodies. The percentage of subjects with IgA antibodies declined for the group aged over 60 yr.

Mean absorbance values for each group were also determined (Figs1 and 2) and showed a steady increase up to the age of 60 yr and a decline thereafter. The mean IgG absorbance for all subjects was 0.322 with a standard deviation of 0.151, a minimum of 0.000 and a maximum of 0.933. A total of 34 sera had absorbance values that were more than 2 standard deviations from the mean. The mean IgA absorbance for all subjects was 0.234 with a standard deviation of 0.115, a minimum of 0.000 and a maximum of 0.891. A total of 25 sera had absorbance values that were more than 2 standard deviations from the mean. Correlation between IgG and IgA absorbance values [r=0.3923 (P<0.0001)] was statistically significant.

In the second part of the study on symptomatic patients, 83 subjects were included. They had a mean age of 39.4 yr and a male:female ratio of 1.67:1. On histological examination, 69 patients had gastritis; of these 58 had *H. pylori* identified on histology or by a positive urease test, while 13 patients were negative for both. In these 58 patients, gastritis associated with *H. pylori* on biopsy and a positive urease test was found in 48, gastritis associated with *H. pylori* but with a negative urease test was seen in 8, a positive urease test alone was seen in 1 patient and in 1 patient, *H. pylori* were identified on biopsy in the absence of gastritis and urease activity. In 12 of 14 subjects without gastritis, both histology and the urease test were negative.

A total of 49 urease tests were positive, with 42 becoming positive in 15 min and the other 7 within 1 h. There was no significant correlation between time taken for the test to become positive and the severity of infection.

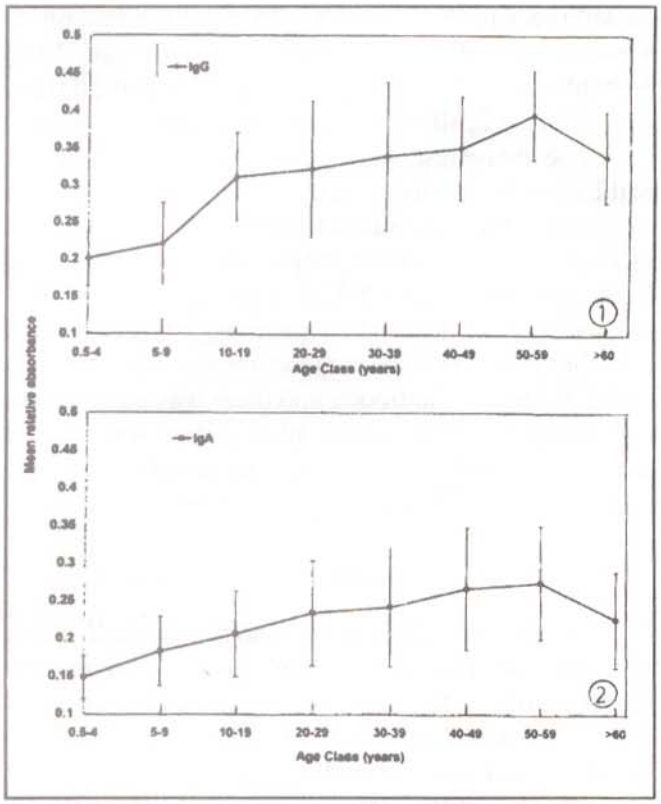
Using either identification of *H. pylori* on biopsy or a positive urease test as the gold standard for evidence of infection with *H. pylori*, sensitivity and specificity were calculated for all 3 classes of antibodies using different cut-off values of optical density, these are shown in Table II. Since IgM cut-off values could not be estimated with cord sera, sensitivity and specificity

Table I. Distribution of the asymptomatic study population by age and sex, and age-specific prevalence of IgG and IgA antibodies to *H. pylori* in asymptomatic subjects

Age range, yr	Number	Male	Female	Percentage positive for	
				IgG	IgA
0.5-4	60	41	19	56.7	43.3
5-9	54	37	17	62.9	64.8
10-19	100	57	43	82.0	81.0
20-29	100	62	38	83.0	75.0
30-39	100	72	28	79.0	84.0
40-49	100	59	41	82.0	86.0
50-59	100	72	28	86.0	87.0
>60	100	71	29	90.0	82.0

Table II. Sensitivity and specificity of determination of antibodies to *H. pylori* in symptomatic patients using histology and the urease test as the gold standard

Antibody	ELISA absorbance	Sensitivity, %	Specificity, %
IgG	>0.400	75.8	72.0
	>0.300	81.0	64.0
IgA	>0.400	46.5	88.0
	>0 300	70.7	72.0
	>0.200	93.1	44.0
IgM	>0.400	55.0	52.0
	>0.300	70.1	32.0



Figs 1 and 2. Mean relative absorbance of *H. pylori* specific IgG and IgA respectively, measured by enzyme immunoassay, for different age classes. The bars represent one standard deviation from the mean.

were calculated using cut-off ODs of 0.300 and 0.400.

In 4 of the 12 patients where the mucosal biopsies were normal, no *H. pylori* were seen and the urease test was negative, IgG and/or IgA levels were elevated above an OD of 0.400. In the 13 patients with gastritis, who showed no *H. pylori* on biopsy and had a negative urease test. IgG levels were elevated above an OD of 0.400 in 4 and IgM levels in 6. In the 2 patients without gastritis but with *H. pylori* on biopsy or a positive urease test, the ODs for both IgG and IgM were above 0.400 and the ODs for IgA were over 0.300. Of the 8 patients with gastritis, *H. pylori* on biopsy and a negative urease test, IgG levels were above an OD of 0.400 in 5 and in an additional 2 patients, OD levels for IgA and IgM were over 0.400.

Varying degrees of inflammation, atrophy and metaplasia were seen in the 69 biopsies with evidence of chronic gastritis. Inflammation was mild or moderate, except in one subject who had severe inflammation associated with heavy *H. pylori* infection with many bacteria seen per high-power field of section examined, a positive urease test and very high levels of IgG. Mild metaplasia was seen in 1 biopsy and moderate metaplasia in 2, all 3 had *H. pylori* infection on biopsy, a positive urease test, high IgG and IgA antibody levels. Mild atrophic changes were seen in 8 subjects and moderate atrophy in 1 subject and all 9 also had evidence of *H. pylori* infection on biopsy and 8 of these 9 had high IgG levels and 5 had high IgA levels. The parameters examined on histopathology were correlated with the OD levels in the enzyme immunoassay for each of the 3 classes of antibody and there was a significant correlation between severity of *H. pylori* infection as assessed on histopathological examination and IgG antibody levels ($P<0.02$).

Discussion

The detection of IgG and IgA antibodies to *H. pylori* is a sensitive and specific method for studying the epidemiology of the infection in populations in whom endoscopy is not indicated[14]. In addition, substitution of serological tests for endoscopy in some classes of patients requires that data be available on the antibody prevalence levels in asymptomatic subjects belonging to the same population in which the test is to be used.

Interpretation of data on the epidemiology is complicated by the variety of assays used to identify a *H. pylori* infection[14,15]. The first generation serological tests, using whole bacteria and bacterial sonicates are sensitive, but not highly specific, resulting in false positive tests. Assays using cell surface proteins, as in the acid-glycine extract used in this study, cell associated antigens and urease preparations are more specific[12-14]. Of the studies reported previously from India, one utilized a first generation commercial kit and tested for IgG and IgA antibodies in 340 subjects[11]. The second utilized a second-generation test, however, the numbers tested (238) were very small[10]. In another study, utilizing a subunit of the urease enzyme as antigen, IgG responses in patients with acid-peptic disease and healthy blood donors were studied[12], but antibody acquisition in children was not studied. In this study, we have used a second generation test and tested more than twice the number of subjects used in the previous 2 studies.

The available data indicate that *H. pylori* infection in India is widespread[10-12,16] and is acquired early in life. The studies on antibody acquisition reviewed here[10,11] and the present study are from the western and southern regions of the country. In children below 5 yr, the study from Mumbai[11] shows the lowest antibody prevalence levels, but by the end of the second decade both IgG and IgA antibody levels are similar to those reported here. In this study, the percentage prevalence of IgG shows a insignificant fall in prevalence level in the age group 30-39 yr, however, the mean OD value (Fig. 1) shows an increase. Similarly, percentage prevalence of IgG antibodies in the age group over 60 yr is 90 per cent, however, the mean OD value shows a decline, reflecting a pattern reported in previous studies. This seems to indicate that determining the mean OD values of each group may provide a truer representation of antibody prevalence in epidemiological studies.

Using the data on the 714 asymptomatic subjects, cut-off values for OD could be established either by using mean ± 2SD, which was not done because of the large SD, or by using the upper limit of normal in each age group, setting the cut-off value at 85 per cent specificity. The highest mean ODs were in the age group 51-60 yr, and for IgG, the upper limit of normal in this age group would be an OD of approximately 0.390 and for IgA, 0.265. In this prospective study on patients with

a pre-endoscopic diagnosis of dyspepsia a diagnosis of 58 *H. pylori* infection was made on the basis of histopathology and the urease test in 48, by histopathology alone in 9 and by the urease test alone in 1. If an OD of 0.390 is used as the cut-off for IgG, 44 of these patients could have been diagnosed without the use of an invasive procedure. The inclusion of IgA OD values of above 0.265 would have identified an additional 6 patients with *H. pylori*, reducing the need for endoscopy further.

Using the upper limit of the normal cut-off values resulted in false positive results in 3 patients with normal histology and a negative urease test, 2 had elevated IgG and 1 had elevated IgA levels. IgG and IgA levels were also elevated in 4 and IgM levels in 6 of the 13 patients with gastritis, with negative histology for *H. pylori* and negative urease test. However, in all biopsy based tests, sampling error can occur, due to patchy colonization and also because the progression of inflammation to atrophy and metaplasia may result in conditions unfavourable for growth of the bacterium[17].

In conclusion, although *H. pylori* is a mucosal infection, in most cases a constant systemic immune response is induced and measurement of IgG antibodies appears to be a sensitive and specific test to diagnose infection, even in a population with a high background infection rate. However, if serology is used to avoid invasive diagnostic techniques in such a population, the test needs careful evaluation and determination of cut-off values, based on the antibody prevalence in the general population.

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