Seroprevalence of Immunoglobulin G, M, and A Antibodies to *Helicobacter pylori* in an Unselected Danish Population

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The seroprevalences of increased levels of immunoglobulin G (IgG), M (IgM), and A (IgA) antibodies to *Helicobacter pylori* were assessed by enzyme-linked immunosorbent assay techniques in 3,589 Danes who participated in a population study in Copenhagen County in 1982. A total of 33.9% of the study population had one or more classes of increased antibodies to *H. pylori*. Increased levels of IgG, IgM, and IgA antibodies to *H. pylori* were seen in 25.9% (95% confidence interval (CI) 24.5–27.3), 4.5% (95% CI 2.2–7.0), and 12.0% (95% CI 10.9–13.1) of the participants, respectively. Women were significantly more likely than men to be seropositive for IgM antibodies (Mantel-Haenszel summary odds ratio = 1.85, 95% CI 1.34–2.57). Seropositivity for IgM antibodies to *H. pylori* was found less often with increasing age. An IgG antibody response was not seen in 23.7% of cases with overall increased antibodies to *H. pylori*. Increased levels of IgG or IgA antibodies were more frequent in people with a history of peptic ulcer disease. Seroprevalences of increased *H. pylori* antibodies are high in unselected populations. Primary *H. pylori* infections are contracted at all ages, but infection rates decline with age. Inclusion of measurements of IgA and IgM antibody levels in future screening for *H. pylori* may improve the diagnostic sensitivity of serologic analyses. Am J Epidemiol 1996;143:1157–64.

*Helicobacter pylori*; immunoglobulins; peptic ulcer; prevalence; seroepidemiologic methods

For the past 10 years, research into gastroduodenal disease has focused on the role of *Helicobacter pylori* infection. Today it is generally accepted that *H. pylori* is involved in the etiology of chronic gastritis, peptic ulcer disease, and gastric cancer (1–5). Several seroepidemiologic studies have shown that *H. pylori* infections prevail in selected populations (6–9). Primary infections are rarely seen, and very little data currently exist on the epidemiology of this condition (6, 7). Nevertheless, to establish guidelines for prophylactic measures against this bacterium and to improve treatment, it is important to examine the epidemiology of acute *H. pylori* infection as well as chronic infection in unselected populations.

Use of noninvasive methods of detecting *H. pylori* is imperative when screening an unselected population. Serologic tests offer high sensitivity and specificity (2, 10–13); furthermore, simultaneous measurement of serum immunoglobulin G (IgG), M (IgM), and A (IgA) antibodies to *H. pylori* can be used to determine the prevalence of both acute and chronic infection (14, 15). Recent progress in preparing more specific low molecular weight antigens has significantly reduced the risk of cross-reaction with other bacteria and has further improved the sensitivity of the IgG enzyme-linked immunosorbent assay (ELISA) (12).

An extensive review of *H. pylori* epidemiology recently noted that several questions on the natural history of *H. pylori* infection remain unanswered (2). To gain insight into the epidemiology of acute and chronic *H. pylori* infection, we assessed the seroprevalences of increased levels of IgG, IgM, and IgA antibodies to *H. pylori* in a large unselected population. Seroprevalences were compared with demographic variables and self-reported ulcer occurrence. The impact of changing cutoff points for IgG seropositivity was also examined.
MATERIALS AND METHODS

Study population and acquisition of serum samples

In 1982, an age- and sex-stratified sample consisting of 4,807 men and women born in the years 1922, 1932, 1942, and 1952 (i.e., aged 30, 40, 50, and 60 years) and residing in the western part of Copenhagen County was drawn from the Danish Civil Registration System, in which all persons living in Denmark are registered by a unique 10-digit number. The distribution of sex, age, occupation, and marital status in the sampling area was compared with Danish national statistics to ensure sample validity. Overrepresentation of unskilled workers and younger people (aged <50 years) was observed in the sample in comparison with Copenhagen County as a whole. Compared with the entire country, there was minor underrepresentation of workers employed in agriculture, horticulture, and fishery, and there were fewer self-employed people and unskilled workers.

All members of the sample were invited to undergo a general health examination through a standardized letter containing information about the project. Also enclosed was a questionnaire on the sample member’s medical history, to be completed in advance (16). Sample members were asked to report whether, prior to study entry, they had ever had an ulcer diagnosed by radiography, endoscopy, or surgery. Statements of ulcer disease were substantiated by scrutinizing medical records (17). Repeated entreaties were made in cases of nonresponse.

After 226 persons of foreign extraction were excluded, the sample size was reduced to 4,581 Caucasian individuals. The response rate was 78.8 percent (3,608/4,581). Between November 1982 and February 1984, serum samples from 3,589 respondents were obtained and stored at a temperature of -20°C. In June 1993, the sera were thawed and analyzed.

The project was approved by the Regional Research Ethics Committee of Copenhagen County.

Antibody detection by ELISA

IgG, IgM, and IgA antibodies to H. pylori were measured in duplicate by indirect ELISA. IgA and IgM antibodies directed against heat-stable H. pylori antigens (11) and IgG antibodies to a low molecular weight fraction of H. pylori antigens (12, 18) were measured as previously described (19). In brief, the selected strain of H. pylori (CH 20429) was grown for 24–48 hours, harvested in phosphate-buffered saline (pH 7.4), and centrifuged at 6,000 × g for 30 minutes. For preparation of heat-stable antigens, a suspension of 0.5 g wet weight H. pylori per ml of phosphate-buffered saline with Triton X100 (Serva Fine Biochemica, Heidelberg, Germany) was ultrasonicated, boiled for 2 hours, and centrifuged. The supernatant was filtered through a 0.2-μm pore size filter. For low molecular weight antigen preparations, a suspension of 0.5 g wet weight H. pylori per ml of phosphate-buffered saline was ultrasonicated and filtrated through filters with molecular weight cutoffs at 100 kDa and 30 kDa. Microtiter plates were coated overnight with the antigen preparations. Serum samples were diluted at 1 : 800 for H. pylori IgG antibody detection and 1 : 100 for detection of IgA and IgM antibodies. After 1 hour of incubation at room temperature, the plates were washed five times. Rabbit antibodies to human IgG, IgM, and IgA (DAKO, Copenhagen, Denmark) conjugated with horseradish peroxidase were diluted at 1 : 1,000 and added to each well. The plates were incubated for 1 hour at room temperature and washed five times, and the enzyme activity was detected using the ortho-phenyle-diamine dihydrochloric acid-hydrogen peroxidase system. After 20 minutes, the chromogenic reaction was stopped with sulfuric acid and the absorbance was read in a photometer at 492 nm. The amount of antibody was expressed in ELISA units (EU), which are absorbance values corrected for day-to-day and plate-to-plate variation (12).

Cutoff points and interpretation of antibody distributions

Based on measurements of serum samples from patients with dyspepsia and known H. pylori status, IgG, IgM, and IgA antibody levels below 100 EU are considered negative (11, 12). Upper cutoff points for IgG antibody seropositivity were assigned at ≥200 EU and ≥400 EU. Cutoff points for IgA and IgM antibody seropositivity were set at ≥100 EU and ≥200 EU, respectively. Borderline cases were those who had antibody levels between 100 EU and the upper cutoff point. The presence of an increased level of IgM antibodies only was interpreted as a serologic sign of primary H. pylori infection. Concomitantly increased levels of IgG and IgM antibodies were assumed to reflect reactivation or reinfection/superinfection with H. pylori.

Statistical methods

The SPSS statistical package for Windows (20) and the SYSTAT package for the Macintosh computer (21) were employed. To allow for cross-tabulations, we transformed the data onto nominal scales according to seroprevalence. Risk estimates were expressed as relative risks. When data were stratified according to sex or age, a summary odds ratio was calculated as

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proposed by Mantel and Haenszel (22). Ninety-five percent confidence intervals for odds ratios and relative risks were computed by the method of Miettinen (23). Testing for trend was used to detect any age trends in seroprevalences. Ninety-five percent confidence intervals for proportions were obtained from the binomial distribution.

RESULTS

IgG antibodies to \textit{H. pylori}

\textit{Increased levels of IgG antibodies (IgG seroprevalence).} The prevalence of increased levels of IgG antibodies to \textit{H. pylori} was 35.1 percent at a cutoff point of ≥200 EU and 25.9 percent at a cutoff point of ≥400 EU (table 1). IgG seroprevalences increased significantly with age at both cutoff levels (table 1). No overall sex differences in IgG seroprevalences were seen (cutoff point of ≥200 EU: Mantel-Haenszel summary odds ratio (OR\textsubscript{M-H}) = 1.01, 95 percent confidence interval (CI) 0.87–1.17; cutoff point of ≥400 EU: OR\textsubscript{M-H} = 1.08, 95 percent CI 0.88–1.22). However, age-specific analyses showed that significantly more 50-year-old women than men had increased levels of IgG antibodies (relative risk = 1.18, 95 percent CI 1.02–1.37).

Changing the cutoff point for IgG seropositivity caused comparable changes in IgG seroprevalence in all sex and age groups. Numbers of borderline cases did not vary between age groups (table 1).

\textit{IgG antibody response in persons with increased IgG, IgM, or IgA antibodies.} Figures 1 and 2 show the associations between antibody classes in 1,217 people with increased antibody levels to \textit{H. pylori}. Levels of IgG antibodies were increased in 76.3 percent (929/1,217) of all cases with increased antibody levels.

<table>
<thead>
<tr>
<th>IgG (cutoff ≥ 400 ELISA units) (n = 471)</th>
<th>IgA (cutoff ≥ 100 ELISA units) (n = 234)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55.2% (n = 343)</td>
<td>55.2% (n = 234)</td>
</tr>
<tr>
<td>17.4% (n = 108)</td>
<td>17.4% (n = 108)</td>
</tr>
<tr>
<td>1.9% (n = 12)</td>
<td>1.9% (n = 12)</td>
</tr>
<tr>
<td>1.6% (n = 10)</td>
<td>1.6% (n = 10)</td>
</tr>
<tr>
<td>5.2% (n = 32)</td>
<td>5.2% (n = 32)</td>
</tr>
</tbody>
</table>

\textit{FIGURE 1.} Distribution (%) of increased levels of immunoglobulin G (IgG), M (IgM), and A (IgA) antibodies to \textit{Helicobacter pylori} in 621 Danish men with overall increased antibody levels, 1982. ELISA, enzyme-linked immunosorbent assay.

\textit{IgM antibodies to \textit{H. pylori}}

\textit{Increased levels of IgM antibodies (IgM seroprevalence).} A total of 4.5 percent (162/3,589) of the study population had increased levels of IgM antibodies to \textit{H. pylori}. IgM seroprevalence declined significantly with age in both sexes (table 2). Women were more likely than men to have increased IgM antibody levels (OR\textsubscript{M-H} = 1.85, 95 percent CI 1.34–2.57). Age-specific analyses showed that this sex difference was significant only in persons younger than age 60 years. The prevalence of primary infection, characterized by increased IgM antibodies only, was 3.8 percent (n = 34/905) in 30-year-olds and 0.4 percent (n = 3/848) in 60-year-olds. The

\textbf{TABLE 1.} Distribution of Immunoglobulin G (IgG) antibodies to \textit{Helicobacter pylori} according to age and cutoff point for IgG seropositivity in 3,589 Danes, 1982

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No</th>
<th>IgG-seronegative (cutoff &lt;100 EU)</th>
<th>IgG-seropositive*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>30</td>
<td>905</td>
<td>70.2</td>
<td>635</td>
</tr>
<tr>
<td>40</td>
<td>927</td>
<td>53.2</td>
<td>493</td>
</tr>
<tr>
<td>50</td>
<td>909</td>
<td>54.8</td>
<td>498</td>
</tr>
<tr>
<td>60</td>
<td>848</td>
<td>42.2</td>
<td>358</td>
</tr>
<tr>
<td>Crude rate</td>
<td>3,589</td>
<td>55.3</td>
<td>1,984</td>
</tr>
<tr>
<td>95% CI†‡</td>
<td>53.7–56.9</td>
<td>33.5–36.7</td>
<td>24.5–27.3</td>
</tr>
</tbody>
</table>

* Test for trend with age: both significant at \( p < 0.001 \).
† EU, ELISA units (plate variation-corrected absorbance values); CI, confidence interval.
‡ Confidence intervals were obtained from the binomial distribution.

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IgG antibodies to *H. pylori*

Increased levels of IgG antibodies (IgG seroprevalence). The prevalence of increased levels of IgG antibodies to *H. pylori* was 12.0 percent (429/3,588) (table 3). A predominance of men among IgG-seropositive people did not reach statistical significance (OR \(_{M-H} = 1.16, 95\% \text{ CI} 0.95-1.43\)). IgG seroprevalence did not vary significantly with age. However, IgA seroprevalence was significantly lower in 30-year-olds than in other age groups (persons aged 30 years vs. other age groups: relative risk = 0.70, 95\% CI 0.55-0.91).

IgA antibodies to *H. pylori*

Increased levels of IgA antibodies (IgA seroprevalence). The prevalence of increased levels of IgA antibodies to *H. pylori* was 12.0 percent (429/3,588) (table 3). A predominance of men among IgA-seropositive people did not reach statistical significance (OR \(_{M-H} = 1.16, 95\% \text{ CI} 0.95-1.43\)). IgA seroprevalence did not vary significantly with age.

**Self-reported ulcer occurrence and *H. pylori* antibody levels**

The associations between IgG, IgM, and IgA seroprevalences and self-reported ulcer occurrence are shown in table 4. The likelihood of a reported history of peptic ulcer disease was significantly increased in people who had increased levels of IgG or IgA antibodies. IgM seroprevalence was not associated with self-reported ulcer occurrence.

In total, 25.9 percent of those reporting a history of peptic ulcer disease had unelevated levels of IgG antibodies to *H. pylori*. Likewise, 84.6 percent and 80.1
TABLE 3. Prevalence of increased serum levels of immunoglobulin A (IgA) antibodies to *Helicobacter pylori*, according to age and sex, in 3,588* Danes, 1982

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No</th>
<th>Overall seroprevalence of increased IgA antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>30</td>
<td>451</td>
<td>454</td>
</tr>
<tr>
<td>40</td>
<td>471</td>
<td>456</td>
</tr>
<tr>
<td>50</td>
<td>461</td>
<td>448</td>
</tr>
<tr>
<td>60</td>
<td>449</td>
<td>398</td>
</tr>
<tr>
<td>Crude rate</td>
<td>1,832</td>
<td>1,756</td>
</tr>
</tbody>
</table>

95% CI, †

9.3–12.2

* IgA antibody analyses were performed on only 3,588 serum samples.
† CI, confidence interval.
‡ Confidence intervals were obtained from the binomial distribution.

percent of peptic ulcer cases were IgM- and IgA-seronegative, respectively.

**DISCUSSION**

The present study is distinguished by its assessment of coexisting levels of IgG, IgA, and IgM antibodies to *H. pylori* in serum samples obtained from a large unselected population. It could nevertheless be argued that the applicability of this study is impaired by the fact that *H. pylori* infections were not verified by other diagnostic methods. However, we think that noninvasive detection methods are imperative when screening unselected populations for *H. pylori*. Although the

TABLE 4. Seroprevalence of immunoglobulin G (IgG), M (IgM), and A (IgA) antibodies to *Helicobacter pylori*, according to self-reported ulcer occurrence, in 3,589 Danes, 1982

<table>
<thead>
<tr>
<th>Antibody*</th>
<th>Self-reported ulcer</th>
<th>No ulcer</th>
<th>RRT† (95% CI)</th>
<th>Self-reported ulcer</th>
<th>No ulcer</th>
<th>RRT† (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>Seronegative</td>
<td>26.4</td>
<td>57.7</td>
<td>24.6</td>
<td>56.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Borderline</td>
<td>12.9</td>
<td>19.5</td>
<td>19.7</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seropositive</td>
<td>60.7</td>
<td>22.8</td>
<td>55.7</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.38</td>
<td>(1.99–2.86)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>Seronegative</td>
<td>85.7</td>
<td>65.2</td>
<td>82.0</td>
<td>78.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Borderline</td>
<td>12.9</td>
<td>11.3</td>
<td>8.2</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seropositive</td>
<td>1.4</td>
<td>3.5</td>
<td>9.8</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.41</td>
<td>(0.11–1.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA‡</td>
<td>Seronegative</td>
<td>82.0</td>
<td>87.8</td>
<td>75.4</td>
<td>69.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seropositive</td>
<td>18.0</td>
<td>12.2</td>
<td>24.6</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.56</td>
<td>(1.04–2.34)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Seronegativity was set at <100 ELISA units (EU). Seropositivity for IgG, IgM, and IgA was set at ≥400 EU, ≥200 EU, and ≥100 EU, respectively.
† RRT, relative risk; CI, confidence interval.
‡ Risk was calculated for reported ulcers in seropositive persons versus reported ulcers in seronegative persons.
§ n = 139 for men, since results of IgA analysis were not available for one male participant who reported an ulcer.
urea breath test has some advantages over serologic analysis, it is expensive and time-consuming. On the other hand, serologic analysis offers high sensitivity and specificity (2, 10–13). Sobala et al. (24) recently suggested that serologic diagnosis is sufficient to detect H. pylori infection and that it may replace endoscopy-based procedures in younger patients in whom gastric cancer is unlikely.

The distribution of IgG seroprevalences found in this study is consistent with the findings of previous reports from developed countries (6–9, 25). Likewise, the lack of a sex difference and the age-related increase in IgG seroprevalence have been well described previously (9, 11, 26, 27). The finding that seroprevalences in an unselected population are comparable to prevalence rates found in dyspeptic individuals speaks against there being a relation between H. pylori infections and upper gastrointestinal tract symptoms (28, 29). The present data do not provide new insight into the rather controversial question of whether age-related increases in IgG seroprevalence are due to a cohort phenomenon or a constant infection rate (6, 7, 30). Nevertheless, our findings on IgM seroprevalences do suggest that H. pylori infection can be acquired at all ages. It is also notable that the increase in IgG seroprevalence with age was found to be unaffected by the choice of cutoff point for seropositivity.

The numbers of people with borderline IgG antibody levels varied little between age groups. Since IgG antibodies have been shown to decline to borderline levels when H. pylori is eradicated (31–33), this finding may indicate that spontaneous eradication of H. pylori is rare in adults under the age of 60 (34).

The diagnostic specificity of the ELISA technique is highly dependent on the choice of H. pylori antigens. Previous studies have most often used crude or semicrude antigens containing whole cells, sonicates, and acid-glycine extracts. However, nonspecific cross-reactions are frequently seen in these assays. Low molecular weight antigens are more specific than crude and semicrude antigens in detecting H. pylori IgG antibodies (12, 13). Indeed, to our knowledge, this study is the first large-scale study to have used low molecular weight antigens to detect serum IgG antibodies to H. pylori.

The sera used in this study had not previously been thawed. In our experience, as well as in that of others (7), long-term storage of sera at −20°C does not affect the validity of IgG antibody measurements by ELISA. Nevertheless, the question of whether long-term storage affects IgA and IgM antibody measurements remains unresolved.

Few studies have examined the seroprevalence of IgM antibodies to H. pylori in unselected populations. Kosunen et al. (31) reported IgM seroprevalence in 144 H. pylori culture-positive Finns using a different antigen and a cutoff point of ≥150 EU for seropositivity. The IgM seroprevalence found was slightly lower than the values seen in the present study. This is surprising, since Kosunen et al. examined a selected population that was suspected of having gastric disease a priori. IgM antibodies are usually directed against the bacterial flagella. Although a high cutoff point (≥200 EU) was applied in the present study to reduce the risk of cross-reactions with the flagella antigens of Campylobacter jejuni (35, 36), we cannot rule out the possibility of false-positive results.

To the best of our knowledge, no previous study has reported sex differences in IgM seroprevalence. The finding that IgM seroprevalence was significantly higher in women than in men in three independent age groups in our study is intriguing and calls for further research. Increased levels of IgM antibodies alone were found less often with increasing age, while concomitantly increased IgM and IgG antibody levels showed no significant age variations. This finding indicates that the overall decrease in IgM seroprevalence with age is mostly due to decreasing rates of primary infection; this is in agreement with recent results from Nova Scotia in Canada (30).

One third (1,217/3,589) of our study population was seropositive for IgG, IgA, or IgM. In this subgroup, 76.3 percent had increased levels of IgG antibodies, whereas the remaining 23.7 percent had increased IgA antibodies only (14.8 percent), increased IgM antibodies only (7.6 percent), or concomitantly increased IgA and IgM antibody levels (1.2 percent). Consequently, we suggest that inclusion of IgA and IgM antibody measurements in future serologic H. pylori screening may improve diagnostic sensitivity considerably. Because the results of our serologic tests were not substantiated by a complementary diagnostic test, this implication must await further research.

Lifetime ulcer prevalence in the study population was 5.5 percent—i.e., 7.6 percent and 3.5 percent in men and women, respectively (17). A high number of seronegative people reported a history of peptic ulcer disease. These ulcers were probably caused by other factors such as tobacco smoking, ingestion of nonsteroidal antiinflammatory drugs, or hereditary predisposition. Alternatively, former H. pylori infections may have been eradicated spontaneously or in connection with antimicrobial therapy (34). The relation between IgM seroprevalence and self-reported ulcer occurrence was weak. Levels of IgM and IgA antibodies are not consistently increased in peptic ulcer disease (37, 38), and the current data indicate that an increased level of IgG antibodies to H. pylori is probably the
most reliable predictor of prior and current peptic ulceration.

In summary, our data show that the seroprevalence of IgG antibodies to H. pylori in an unselected Danish population is comparable to prevalences previously reported from other developed countries. Findings on IgM seroprevalence indicate that women have a higher risk of primary H. pylori infection than do men in adult life. H. pylori can be contracted throughout life, but the prevalence of primary infection seems to decline with age.

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