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# Seroprevalence of *Helicobacter Pylori* Infection in Dyspeptic Patients

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## ABSTRACT

The objective of the study was to assess the effect of age on the seroprevalence of *Helicobacter* (*H.*) *pylori* infection in dyspeptic patients. The results obtained in the patient group were compared with findings on the seroprevalence of *H. pylori* infection in 2051 blood donors. Serum samples were tested by the commercial ELISA and CFT assays according to manufacturer's instructions. The mean seroprevalence of *H. pylori* infection as determined by ELISA/CFT was 64.0%/51.7% in the group of blood donors and 92.3%/89.5% in the group of dyspeptic patients. Study results indicated a higher prevalence of *H. pylori* infection in dyspeptic patients as compared with blood donors in all age groups. In the patient Sgroup, *H. pylori* seroprevalence was not age dependent.

**Key words:** seroprevalence, *Helicobacter pylori*, dyspeptic patients, enzyme-linked immunosorbent assay (ELISA), complement fixation test (CFT)

## Introduction

Epidemiological studies conducted all over the world have defined *Helicobacter* (*H.*) *pylori* infection as one of the currently most common infections in humans<sup>1-5</sup>. However, many issues related to the onset, dissemination and rate of infection in different populations yet remain to be elucidated<sup>4-7</sup>. Effects of particular factors of the microorganism virulence and characteristics of different human populations in terms of clinical picture, infection pattern and its possible late sequels have been continuously investigated.

Man is the only recipient host for *H. pylori*. However, gastric colonization with a *H. pylori*-like bacterium has also been detected in cat and swine<sup>8-10</sup>. Epidemiological studies conducted to date point to two routes of *H. pylori* infection dissemination, fecal-oral and oral-oral. *H. pylori* was isolated from stool samples of children (Gambia) and adults (Great Britain)<sup>11,12</sup>. The bacterium was also isolated in dental plaque<sup>13</sup>. The rate of oral cavity colonization with *H. pylori* is very low in industrialized west-

ern countries, diminishing the role of oral-oral transmission (kissing, food)<sup>14,15</sup>.

Besides age, the prevalence of *H. pylori* infection is mostly influenced by the socioeconomic status (over-population and poverty, especially in childhood; water contamination; multi-member and poor families living in cramped conditions) and place of residence<sup>16</sup>. In Great Britain, the prevalence of *H. pylori* infection was considerably greater in adults having lived in multi-member families in their childhood. The prevalence was even greater in the individuals that used to share bed with one or more family members or having lived in a household without running water during childhood<sup>17</sup>. The reason for these findings most probably lies in long-term close interpersonal contacts along with low hygiene conditions<sup>18</sup>. In some countries (Peru), contaminated water supply may be the source of *H. pylori* infection<sup>19</sup>.

Pets (e.g., cat) probably are another possible source of infection<sup>20</sup>. *H. pylori* infection may also be transmitted

on endoscopic examination; transmission of the infection was recorded in 1–3% of endoscopic examinations with manual endoscope washing, but not in endoscopy units using mechanical endoscope cleaning and sterilization<sup>21</sup>. Some professions<sup>22,23</sup>, among them health professionals, GI endoscopy laboratory staff in particular, are at an increased risk of *H. pylori* infection<sup>24,25</sup>.

In western countries, the prevalence of *H. pylori* infection can generally be characterized as follows: (a) *H. pylori* is present in approximately 20% of individuals below 40 and in 50% of individuals over 60 years of age; (b) *H. pylori* infection is a rare or unusual disease in small children; (c) the lower the socioeconomic status, the higher the prevalence of *H. pylori* infection; and (d) the high prevalence of *H. pylori* infection in some areas of industrialized countries is consequential to the increased immigration of infected persons<sup>4,5,26</sup>. Current studies indicate low incidence of *H. pylori* infection in children and young individuals. The high incidence of the infection in adults is consequential to long-standing and permanent infection acquired in childhood, along with the impact of low socioeconomic conditions<sup>27</sup>.

In contrast to industrialized countries, in the majority of developing countries (Brazil, Asia, Africa, and East Europe) *H. pylori* infection is found in almost all adult individuals. In these regions, the infection is already present in 10% of children aged 2–8; in adolescence, the incidence rises to 85–95%<sup>1,2,28,29</sup>.

Seroprevalence of *H. pylori* infection was also assessed in dyspeptic patients. Some authors report a comparable seroprevalence of *H. pylori* infection in symptomatic and asymptomatic subjects<sup>2,5–7,30–32</sup>, whereas others found it to be higher in dyspeptic subjects as compared with blood donors<sup>33–35</sup>.

Considering the increasing seroprevalence of *H. pylori* infection with age and variable results reported in dyspeptic patients, we embarked upon the present study to assess the effect of age on the seroprevalence of *H. pylori* infection in patients with dyspeptic complaints. The results recorded in the group of dyspeptic patients were compared with those obtained in blood donors serving as a control group of normal population.

## Materials and Methods

### Serum samples

The presence of specific *H. pylori* antibodies was determined in 2701 serum samples, including 650 patients with clinical indications for gastric and duodenal endoscopy (pain in upper abdomen with dyspeptic symptoms) and 2051 healthy subjects (blood donors). Patients were recruited from two hospitals: Sveti Duh General Hospital from Zagreb and Karlovac General Hospital from Karlovac. Out of 650 patients, 500 (300 male and 200 female, mean age 55.3 years) regularly attended Endoscopy Laboratory, Sveti Duh General Hospital, whereas the remaining 150 patients (135 male and 15 female,

**TABLE 1**  
BASELINE PATIENT CHARACTERISTICS

Patient group (institution)	N	Mean age (yrs)
Sveti Duh General Hospital	500	55.3
Male	300	54.9
Female	200	55.5
Karlovac General Hospital	150	42.5
Male	135	42.8
Female	15	41.5
Total	650	52.9

mean age 42.5 years) attended Endoscopy Laboratory, Karlovac General Hospital (Table 1).

Serum samples were stored at –20 °C until analysis. The study was conducted at the two hospitals during the 2001–2006 period.

### Questionnaire

All study subjects filled out a questionnaire containing data on age and upper abdominal discomforts. Data on dyspeptic complaints and/or treatment for gastric or duodenal peptic disease were only entered by the patient group.

### Detection of *H. pylori* antibodies

Serum samples were tested with commercial enzyme-linked immunosorbent assay (ELISA; Eurospital, Trieste, Italy) and complement fixation test (CFT; Institute Virion, Zurich, Switzerland). The tests were performed according to the manufacturer's instructions. Borderline test values were established in line with the manufacturer's instructions to interpret the results obtained.

ELISA: each serum sample diluted 1:200 was applied onto a microtiter plate with previously bound *H. pylori* antigen. The antigen-antibody complex was proven by sheep antihuman IgG antibodies labeled with alkaline phosphatase and incubated with chromogen substrate. The substrate absorption was determined by ELISA reader (Multiscan, Titertek, MCC/340, Finland). An index of IgG antibodies equal or higher than 40% was considered as positive result.

CFT: complement fixation antibodies (IgM, IgG) were proven by *H. pylori* strain Lior type 1. Each serum sample was diluted with a 1:10 Veronal buffer and incubated for 30 minutes at 56 °C to inactivate the complement present in the serum. Then serum samples as well as positive and negative serum controls were diluted from 1:10 to 1:160, with the addition of the respective antigen and complement dilution. The test included controls to detect anticomplement activity in each sample tested as well as the control for the complement used (0.5, 1.0, 1.5 and 2.0 units of complement). CFT result was assessed on the basis of hemolysis inhibition. The inhibition of 50% or more was considered positive, indicating the presence of anti-

bodies in the respective dilution. Antibody titer of less than 1:30 was considered negative.

### Statistics

The  $\chi^2$ -test for dependent and independent samples, and the test of proportions were used. Statistical analysis was done by use of the Microstat software. Statistical significance was set at  $p < 0.05$ .

### Results

In the study group of 650 dyspeptic patients divided according to age groups, neither  $\chi^2$ -test nor the test of proportions yielded any statistically significant difference between the seroprevalence of *H. pylori* infection as determined by the ELISA and CFT (Table 2).

ELISA and CFT were used to test 2051 serum samples obtained from blood donors living in the Zagreb area. The  $\chi^2$ -test showed no statistically significant difference between the seroprevalence of *H. pylori* infection as determined by ELISA and CFT in serum samples of blood donors divided according to age groups (Table 3). However, the test of proportions indicated a statistically significantly higher seroprevalence of *H. pylori* infection by ELISA as compared with CFT in all age groups ( $\leq 29$ ):  $Z = 9.13$ ,  $p < 0.001$ ; (30–39):  $Z = 3.58$ ,  $p < 0.001$ ; (40–49):  $Z = 3.90$ ,  $p < 0.001$ ; and ( $\geq 50$ ):  $Z = 6.98$ ,  $p < 0.001$ ).

The proportions tested according to age groups revealed an increase in the proportion of positive results with age both by ELISA ( $\leq 29/30-39$ ):  $Z = 2.17$ ,  $p < 0.01$ ; (30–39/40–49):  $Z = 1.58$ ,  $p < 0.05$ ; and (40–49/ $\geq 50$ ):  $Z = 3.78$ ,  $p < 0.001$ ) and by CFT ( $\leq 29/30-39$ ):  $Z = 6.79$ ,  $p < 0.001$ ; (30–39/40–49):  $Z = 2.54$ ,  $p < 0.05$ ; (40–49/ $\geq 50$ ):  $Z = 5.49$ ,  $p < 0.001$ ). The recorded seroprevalence proportions according to age groups pointed to a gradual rise in the proportion of positive infection with age only in the control group of blood donors. In the study group of dyspeptic patients, however, in spite of the higher prevalence of *H. pylori* infection, age had no major impact on the seroprevalence proportion. Both ELISA and CFT methods yielded a statistically significantly higher number of positive results in all patient age groups as compared with the respective age groups of blood donors: ELISA ( $\leq 29$ ):  $Z = 3.29$ , (30–39):  $Z = 4.78$ , (40–49):  $Z = 6.72$ , and ( $\geq 50$ ):  $Z = 6.12$  ( $p < 0.001$  all) and CFT ( $\leq 29$ ):  $Z = 4.63$ , (30–39):  $Z = 6.88$ , (40–49):  $Z = 8.09$ , ( $\geq 50$ ):  $Z = 4.93$  ( $p < 0.001$  all) (Table 2 and 3).

### Discussion

Endoscopic approach is a basis of all invasive diagnostic methods with targeted biopsy sampling for detection of *H. pylori* infection<sup>36</sup>. Such an approach is not acceptable for epidemiological studies because it is very expensive and uncomfortable for the patient. Of the noninvasive methods available, serology is the method of choice for epidemiological studies. In addition, antibody detection in serum samples obtained at different times in the

**TABLE 2**  
SEROPREVALENCE OF *H. PYLORI* INFECTION IN DYSPEPTIC PATIENTS ACCORDING TO AGE GROUPS

Age group (yrs)	N	ELISA (+) n (%)	CFT (+) n (%)
$\leq 29^*$	64	52 (81.2)	47 (73.4)
30–39*	101	93 (92.7)	87 (86.1)
40–49*	158	150 (94.9)	147 (93.0)
$\geq 50^*$	327	305 (93.9)	301 (92.0)
Total	650	600 (92.3)	582 (89.5)

N – total number of patients tested, (+) – *Helicobacter pylori* positive patients, ELISA – enzyme-linked immunosorbent assay, CFT – complement fixation test, \* $p < 0.001$  (Table2/Table3)

**TABLE 3**  
SEROPREVALENCE OF *H. PYLORI* INFECTION IN BLOOD DONORS ACCORDING TO AGE GROUPS

Age group (yrs)	N	ELISA (+) n (%)	CFT (+) n (%)
$\leq 29^*$	218	114 (52.2)**	77 (35.3)**
30–39*	743	454 (61.1)**	351 (47.2)**
40–49*	787	510 (64.8)**	421 (53.4)**
$\geq 50^*$	303	235 (77.5)**	211 (69.6)**
Total	2051	1313 (64.0)	1060 (51.7)

N – total number of patients tested, (+) – *Helicobacter pylori* positive subjects; ELISA – enzyme-linked immunosorbent assay, CFT – complement fixation test, \* $p < 0.001$  (Table2/Table3), \*\* $p < 0.001$

course of infection and stored frozen until analysis allows for the characteristics of the course of chronic infection to identify.

Serologic methods are characterized by high specificity, verifying the presence of *H. pylori* infection in seropositive individuals with high certainty, while the high sensitivity of these methods ensures identification of nearly all infected individuals. The serologic methods employed in the present study were previously demonstrated to have sensitivity over 90% and specificity of about 80%<sup>37</sup>.

Serologic methods are preferred for detection of *H. pylori* infection in patients with atrophic gastritis<sup>38–40</sup> and bleeding ulcers<sup>41–43</sup>. The Western blot technique has been increasingly employed to detect *H. pylori* antigen antibodies (VacA, CagA), which point to an increased risk of ulcer disease and gastric adenocarcinoma<sup>44–46</sup>.

Although the seroprevalence rate determined in volunteers or blood donors cannot be considered representative for the respective population at large, such studies point to the proportional seropositivity increase with age<sup>47</sup>. In their study, Babuš *et al.* found the mean seroprevalence of *H. pylori* infection in Croatia to be 60.4% (95% CI 58.7–62.1%)<sup>48</sup>. The rate of infection increases with the population aging, ranging from 51.6% in the

third decade of life through nearly 70% in the sixth decade of life. In our study, the seroprevalence of *H. pylori* infection in blood donors from the City of Zagreb as determined by ELISA (64.0%) exceeded the figure yielded by CFT (51.7%) as well as the mean rate for Croatia (60.4%). The prevalence of *H. pylori* infection was definitely greater in dyspeptic patients (Table 2) than in blood donors (Table 3): 92.3% vs. 64.0% by ELISA and 89.5% vs. 51.7% with CFT. In the group of dyspeptic patients, there was no statistically significant difference in the proportion of *H. pylori* infection positivity according to age groups. Our results confirmed literature reports on the seroprevalence increase with the population aging<sup>30,48,49</sup> and on the higher *H. pylori* seroprevalence in patients with dyspeptic complaints<sup>33–35</sup>. In countries with a high rate of *H. pylori* seroprevalence, i.e. developing countries, there will probably be no statistically significant difference in *H. pylori* seroprevalence between

asymptomatic individuals and dyspeptic patients<sup>6</sup>. Schilling *et al.* found no difference in *H. pylori* seroprevalence according to dyspeptic patient subgroups<sup>35</sup>.

The present study demonstrated the value, reliability and justifiability of the use of complement binding reaction (CFT) and immunoenzyme testing (ELISA) in sero-epidemiological studies. Results of our study support the use of serology as the method of choice in epidemiological studies. Although the seroprevalence determined in blood donors cannot be considered representative for the respective population, the results obtained indicated a proportional age related increase in the rate of seropositivity in the Zagreb population. The prevalence of *H. pylori* infection was found to be greater in dyspeptic patients in all age groups as compared with blood donors. In the former, however, the rate of *H. pylori* seroprevalence was not age dependent.

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## SEROPREVALENCIJA INFEKCIJE *H. PYLORI* U BOLESNIKA S DISPEPTIČNIM TEGOBAMA

### SAŽETAK

Ovim radom željeli smo utvrditi utjecaj životne dobi na seroprevalenciju infekcije *Helicobacter pylori* kod bolesnika s dispeptičnim tegobama. Dobivene nalaze usporedili smo s nalazima seroprevalencije ove infekcije u 2051 dobrovoljnog darovatelja krvi. Uzorci seruma testirani su komercijalnim pripravcima ELISA i CFT prema uputama proizvođača. U skupini dobrovoljnih darovatelja krvi prosječna seroprevalencija infekcije bila je 64,0%/51,7% (ELISA/CFT), a u skupini bolesnika s dispeptičnim tegobama 92,3%/89,5% (ELISA/CFT). Rezultati rada ukazuju na veću učestalost infekcije *H. pylori* u bolesnika nego kod dobrovoljnih darovatelja krvi po svim dobnim skupinama, a u skupini bolesnika je utvrđeno da seroprevalencija *H. pylori* ne ovisi o životnoj dobi.