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Research article

Helicobacter pylori (H pylori) infection in Greece: the changing prevalence during a ten-year period and its antigenic profile

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Abstract

Background: To evaluate changes in H pylori infection prevalence in Greece during a ten-year period, and to examine its antigenic profile.

Methods: Three groups of patients were studied. Group O-87: Banked serum samples of 200 consecutive adult outpatients, from the Hepato-Gastroenterology clinic of a teaching hospital at Athens, collected in 1987. Group O-97: Serum samples of 201 similarly selected outpatients from the same Unit, collected in 1997. Group BD-97: Serum samples of 120 consecutive blood donors from the same hospital, collected in 1997. H pylori IgG antibody seroprevalence was studied by a quantitative ELISA. Antigenic profile was studied by western-blot IgG assay, in 62 IgG positive patients of O-97 and BD-97. Results were analyzed by conventional statistics and multivariate regression analysis.

Results: The H pylori seroprevalence increased with age in the three tested groups. In O-97, seroprevalence did not differ from that in BD-97. On the contrary, there was a significant decrease in seropositivity between O-87 and O-97 (59.5% vs 49.2%, p = 0.039). Multiple regression analysis showed that age over 35 years (OR:3.45, 95% CI:1.59–7.49, p = 0.002) and year of patients' selection – that is 1987 or 1997 – (OR:1.73, 95% CI:1.14–2.65 for 1987, p = 0.010), were independent risk factors of H pylori infection. The seroprevalence of CagA+ and VacA+ strains was 77.4% and 58.5%, respectively, and type I(CagA+/VacA+) strains were significantly more common than type II(CagA-/VacA-) strains (59.7% vs 22.6%, p < 0.001).

Conclusions: During a ten-year period, we found a significant decrease of H pylori infection in Greece and our data support the birth cohort phenomenon as an explanation for the age-dependent increase of H pylori infection. The prevalence of CagA and/or VacA positive strains is relatively high, in a country with low incidence of gastric cancer.

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Background

It is known that H pylori infection occurs mainly in early childhood and the link between the infection and risk factors such as socioeconomic status and living conditions in childhood is well documented [1–3]. Thus, there is a marked difference in the prevalence of H pylori infection between developing and developed countries during early childhood [4]. However, everywhere in the world, serological data have shown that the prevalence of the infection increases with age [5–7]. In particular in Greece, according to a study carried out in the early '90s [8] seroprevalence increased about 10% per 10 years, from 40% in people aged 21–40 years to 77% in those older than 60 years.

Long-term follow-up studies in developed countries, with low rates of H pylori infection, suggest that the age-dependent increase of seropositivity is mainly due to the decreasing rate of childhood infections [9–13], since many studies have proved that during adulthood the rates of seroconversion and seroreversion are almost equal [9,11,14,15]. This phenomenon, is often referred as a "birth cohort phenomenon". In contrast to this theory, there is also evidence suggesting that a continuous risk of acquisition rather than a cohort effect, best explains the age-dependent increase of seropositivity [16]. However, in populations with higher rates of infection than that observed in occidental and Scandinavian countries, there are no data available to evaluate if the cohort phenomenon or the continuous risk of acquisition could better explain the age dependent increase of H pylori infection.

Nevertheless, it has been shown that some H pylori genes (*vacA*, *cagA*) confer different biological properties which could enhance the in vivo pathogenicity of the bacteria [17,18]. Patients infected with cagA-positive strains of H pylori demonstrate enhanced expression of various cytokines [19] and these patients present a higher grade of gastric inflammation and accelerated epithelial damage [20]. Thus, recently, there has been an interest in detecting the H pylori immunophenotype and in particular, the CagA and/or VacA status.

The aim of this study was two-fold: first, to examine the seroprevalence of H pylori infection in two samples of Greek adult population that were collected in 1987 and in 1997, that is ten years apart, in order to find out if the "birth cohort phenomenon" or the continuous risk of acquisition could better explain the age-dependent increase of H pylori seropositivity, in a country of south-east Europe with relatively high rate of H pylori infection; and second, to evaluate the antigenic profile of the H pylori infection (Vag, Cag etc) in a Greek adult population.

Material and Methods

Subjects

The study population included three groups of patients:

Group O-87

Banked serum samples of 200 consecutive adult outpatients, irrespective of their socioeconomic status and the cause of admission, (107 men and 93 women, aged 15–82 years, mean age 44.3 +17 years), from Hepatology Section of the Gastroenterology clinic of 1st Department of Propedeutic Medicine (Athens University School of Medicine, "Laikon" General Hospital). The used serum samples were originally obtained in 1987, for the study of viral hepatitis.

Group O-97

Serum samples of 201 similarly selected outpatients (123 men and 78 women, aged 16–85 years, mean age 45.9 +15.2 years), from the same Section, that were collected in 1997.

Group BD-97

Serum samples of 120 consecutive blood donors from the same hospital (102 men and 18 women, aged 18–62 years, mean age 40.1 +10.8 years), collected in 1997.

Study design and definition of variables

Group O-97 was compared with group BD-97. Subsequently, group O-97 was compared with group O-87. The analyzed variables were age and gender. Both groups (O-87 and O-97) comprised of consecutive outpatients from the same central hospital in Athens. Thus, the demographic data and the average socioeconomic profile of patients could be considered roughly similar in both groups, taking of course into consideration the total development of the socioeconomic and educational level in our country during the last decade.

The antigenic profile of the infection was studied in 62 randomly selected H pylori IgG antibody positive patients of O-97 and BD-97 groups (the first 40 and 22 consecutive H pylori IgG antibody positive samples of O-97 and BD-97 groups, respectively).

Assays

All serum samples were stored at -70°C and had not been thawed before the current analysis, except for the samples of O-87 group which had been thawed once before.

Assay for antibodies to H pylori

H pylori seroprevalence was studied determining IgG antibodies by a quantitative enzyme-linked immunosorbent assay (ELISA, Diasorin Diagnostics Srl-Manufactured by Hycor Biomedical GmbH). The antigen used was extracted by sonication from the H pylori strain NTCC 43054.

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The main protein fractions which predominantly included in H pylori antigen coated to ELISA microtiter plates, were: p120–130, p83–87, p67, p63/66, p50–59, p28–31, p19 and p14. These immunogenic proteins were enriched and purified by a designated developed biochemical procedure. All assays were performed in duplicate and the intra-assay and inter-assay variations were <5%, as was estimated with the positive and negative control sera. Validation of the ELISA used, was performed, at the same period, in 40 consecutive patients of the Endoscopy Unit of the same hospital. Using histology (modified Giemsa) as "gold-standard", the estimated sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of that ELISA were 87%, 94%, 95% and 84%, respectively.

Antigenic profile of H pylori infection

The antigenic profile of the infection was studied using Immunoblot Helicobacter pylori IgG (Microgen GmbH, Germany). This test permits the safe identification and localization of specific antibodies on the solid phase because the antigens are electrophoretically separated. The test results were evaluated according to the manufacturer's instructions and the main detected bands were: p120(CagA), p87(VacA), p62(UreB), p58(HspA&B), p54(FlaA) and p29(UreA). Using histology and/or microbiology as "gold standard", the sensitivity and specificity of this immunoblotting test was 95% and 83%, respectively (Mikrogen molekularbiologische Entwicklungs-Gmbh, Immunoblot Helicobacter pylori IgG, Instructions for use version: GIIBHPE004.DOC).

Statistical analysis

Chi-square test was used to test for an association between H pylori infection and year of patients' selection, by age group and overall. Multivariate logistic regression was used to determine risk factors associated with H pylori infection. A model containing all variables was considered. Variables that did not contribute to the model, based on their Wald statistic, were eliminated and the new model was compared to the old through the likelihood ratio statistic. Variables whose exclusion gave a non-significant likelihood ratio statistic ($p > 0.05$) were omitted from the model.

Results

H pylori seropositivity in O-97 and BD-97 groups (table 1, fig. 1)

In 1997, seroprevalence of H pylori infection in all patients and in the patients' age groups did not differ from that of blood donors Thus in group BD-97, seropositivity was 11% up to 24 years, increased of about 136% per decade up to 44 years and continued to increase at an average of 13% per decade up to 60 years. In group O-97 seropositivity was 14.2% up to 24 years, increased of about 96% per decade up to 44 years and continued to increase at an average of 16.5% per decade up to 64 years.

Prevalence of antibodies in O-87 and O-97 groups of patients (table 1, figure 1)

The prevalence increased with age in both tested groups of patients (O-87 and O-97). More specifically, in 1987, the increase was continuous up to 45–54 years, at an average rate of 26% per decade. After the age of 55 years, a decrease of seropositivity at an average of 10% per decade was observed.

Table 1: Seroprevalence of H pylori antibodies (IgG) in O-87, O-97 and BD-97 groups.

Age	Studied number			H pylori IgG(+) (%)		
(years)	O-87	O-97	BD-97	O-87	O-97	BD-97
15–24	31	21	9	12(38.7)	3(14.2)	1(11.1)
25–34	39	33	30	20(51.3)	12(36.4)	11(36.6)
35–44	31	42	42	21(67.7)	21(50.0)	22(52.4)
45–54	32	43	27	25(78.1)†	23(53.5)†	16(59.2)
55–64	37	43	12	24(64.8)	29(67.4)	8(66.6)
> 65	30	19	–	17(56.7)	11(57.9)	–
Total	200	201	120	119(59.5)‡	99(49.2)‡	58(48.3)

† $p = 0.032$, ‡ $p = 0.039$

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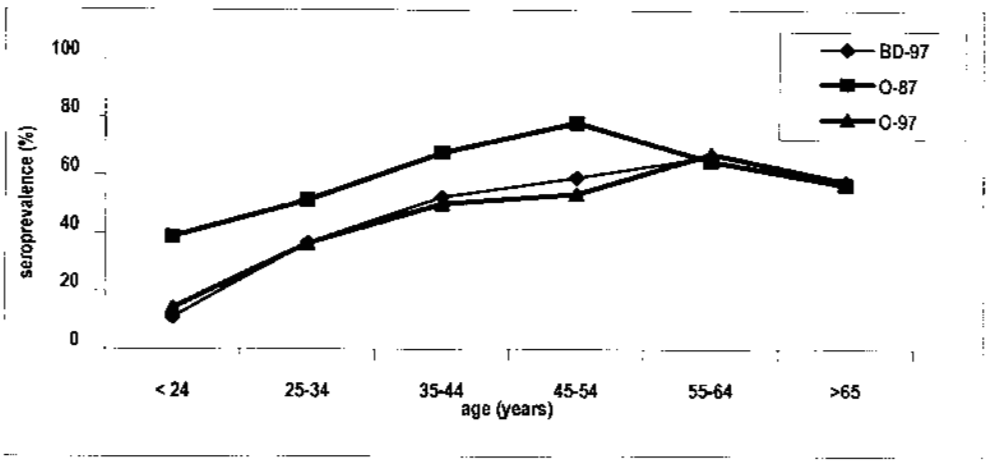

Figure 1
Seroprevalence of H pylori antibodies (IgG) in O-87, O-97 and BD-97 groups.

Table 2: Independent risk factors of H pylori seropositivity in each group of tested patients (O-87, O-97) (multivariate logistic regression analysis).

Variables Age(years)	O-87 Odds ratio	95% CI	p	O-97 Odds ratio	95% CI	p
15 – 24	1			1		
25 – 34	1.68	(0.63, 4.47)	*0.29*	3.34	(0.81, 13.73)	*0.09*
35 – 44	3.28	(1.13, 9.49)	*0.02*	5.54	(1.41, 21.88)	*0.01*
45 – 54	6.05	(1.96, 18.65)	*0.002*	7.12	(1.80, 28.07)	*0.005*
55 – 64	3.28	(1.19, 9.02)	*0.02*	13.51	(3.35, 54.51)	*< 0.001*

In 1997, the prevalence was increasing up to the 55–64 years group, at an average rate of 50% per decade. The average increasing rate was highest for up to 44 years (96% per decade) and continued at an average of 16.5% per decade up to the 55–64 years group. In the oldest age group (>65 years), a decrease of seropositivity of about 14% per decade was noticed.

Logistic regression analysis in the group O-87 with independent variables such as age and gender, showed that age over 35 years was the only independent risk factors of H pylori infection (table 2). Gender was not associated with positivity, but there was a small but insignificant increase of seropositivity in males (OR: 1.24, 95% CI: 0.68–2.25, p = 0.47).

During a ten-year period (1987–1997) the crude H pylori seroprevalence fell significantly by 10% (from 59.5% in group O-87, to 49.2% in group O-97, p = 0.039), and it was constantly lower in each age group (up to 55–64 years) by 15 – 24%.

Logistic regression analysis in all patients (group O-87 and group O-97, 401 patients) with independent varia-

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bles such as age, gender and year of patients' selection (that is 1987 or 1997), showed that age over 35 years and the year 1987 were independent risk factors for H pylori infection (table 3).

Table 3: Independent risk factors of H pylori seropositivity in O-87 and O-97 groups of patients (N:401) (multivariate logistic regression analysis).

Variables *Age(years)*	Odds ratio	95% CI	p
15 – 24	1		
25 – 34	2.02	(0.94, 4.37)	0.073
35 – 44	3.45	(1.59, 7.49)	0.002
45 – 54	5.07	(2.31, 11.11)	< 0.001
55 – 64	5.60	(2.57, 12.19)	< 0.001
> 65	3.49	(1.51, 8.10)	0.004
Period			
1997	1		
1987	1.73	(1.14, 2.65)	0.010

The peak of H pylori seroprevalence differs by one decade in the two tested samples (fig 1). This may suggest that the risk of infection is associated with the year of patients' birth. Indeed, in both groups of patients the highest risk for H pylori seropositivity was found in patients who were born during the decade 1933–1942 in comparison with those born after 1953) (table 4).

The antigenic profile of H pylori infection (table 5)

The seroprevalence of CagA and VacA antigens was 77.4% and 59.7%, respectively. These rates were constant across gender, age and group of patients (O-97 and BD-97). Type I (CagA+/VacA+) strains were significantly more common than type II (CagA-/VacA-) strains (59.7% vs. 22.6%, p < 0.001). The prevalence of the other antigens ranged from 33.9% (FlaA) to 82.2% (UreB).

Discussion

Our findings show that the prevalence of H pylori IgG antibodies increases with age in the three tested groups of patients (O-87, O-97 and BD-97). Provided that the ELISA used had 87% sensitivity and 94% specificity, the results reflect satisfactorily H pylori infection rate. H pylori seroprevalence did not differ between the group O-97 and the healthy controls (group BD-97)(49.2% vs 48.3%, respectively, p = 0.87). On the contrary, comparing the group O-87 with the group O-97, we found a significant decrease of the infection rate in 1997 (59.5% vs 49.2%, respectively, p = 0.039).

The dynamic of infection in 1987 seems to be similar with that seen in the developing countries. Ten years later, in 1997, the picture changed toward what was found in the developed countries. Thus, the crude seroprevalence was reduced about 10% units; multiple regression analysis showed that a person of the O-87 group had a significant higher risk of seropositivity than a comparable person of the O-97 group (OR:1.73, 95% CI: 1.14–2.65, p = 0.010).

As we did not found paired serum samples of the same patient in groups O-87 and O-97 we could not know the rate of serocorversion in our population. But, in both developing and developed countries, there are many studies suggesting that, during adulthood, the rate of seroconversion and seroreversion is almost the same[9,14,15]. Thus, though our data showed that increasing age does increase the risk of H pylori infection, the fact that patients from group O-87 had higher rates of infection than comparable patients from group O-97, supports the "birth cohort phenomenon" as the main cause for the increasing occurrence of the infection seen with age.

Table 4: Decade of birth as an independent risk factor of H pylori seropositivity (multivariate logistic regression analysis).

	1987			1997		
Year of patients' birth	Odds ratio	95% CI	p	Odds ratio	95% CI	p
> 1953	1			1		
1943–1952	2.49	1.01 – 6.14	*0.047*	2.08	1.00 – 4.34	*0.050*
1933–1942	4.54	1.71 – 12.03	*0.002*	3.57	1.67 – 7.62	*0.001*
1923–1932	2.47	1.07 – 5.73	*0.035*	2.87	0.96 – 8.56	*0.058*
< 1923	1.69	0.70 – 4.06	*0.24*	2.08	0.10 – 28.38	*0.704*

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Table 5: Western-blot analysis in 62 H pylori (IgG antibodies) positive persons.

	Studied number		Rate (%)		
	O-97 (N = 40)	BD-97 (N = 22)	O-97	BD-97	Total (N = 62)
p120 (*CagA*)	32	16	80	72.7	77.4
p87 (*VacA*)	25	12	62.5	54.5	59.7
p63 (*UreB*)	33	18	82.5	81.8	82.2
p58 (*HrpA&B*)	32	12	77.5	54.5	71
p54 (*FlaA*)	17	4	42.5	18.2	33.9
p29 (*UreA*)	24	16	60	72.7	64.5

The highest risk of infection in both groups of patients, was found in those born around 1940, that is the period of war deprivation. This finding, in association with the reduction in the infection rate during the studied 10-year period is compatible with the hypothesis that better socioeconomic conditions and improved hygiene have reduced the risk of H pylori infection [4].

In both groups of patients (O-87 and O-97) we found a constant decrease of seropositivity in the oldest age group. This may be due to many factors like spontaneous seroreversion [14], increased antibiotics and NSAIDs consumption [21] and advanced gastric atrophy in association with the natural senility of the immunological system [22].

In this study the prevalence of CagA positive and VacA positive strains of H pylori were 77.4% and 59.7%, respectively; type I strains were significantly more common than type II strains (59.7% vs. 22.6%, $p < 0.001$). According to the Eurogast study group, Greece has low incidence of gastric cancer in comparison with other European countries [23]. It has been suggested that CagA positive infections are more likely to predispose to gastric cancer that the CagA negative ones [24]. Nevertheless, we found a high rate of CagA positive and type I strains of H pylori in our sample. In accordance with previous studies [20,25,26], these data suggest that CagA and/or VacA status are not the only factors that influence gastric cancer rates.

To conclude, a significant decrease of H pylori infections' rate was noticed in Greece during a ten-year period. Even in a country of south-east Europe, with relatively high rate of H pylori infection, our data support the birth cohort phenomenon as an explanation for the age-dependent increase of H pylori infection. The prevalence of CagA and/or VacA positive strains is relatively high for the reported incidence of gastric cancer in Greece.

Competing interests

None declared.

Authors' contributions

PA carried out the immunoassays, participated in its design and sequence alignment and drafted the manuscript. IV participated in its design and coordination. MT participated in the sequence alignment. DK performed the statistical analysis. NK participated in its design and coordination. AA conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

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Effectiveness of two quadruple, tetracycline- or clarithromycin-containing, second-line, Helicobacter pylori *eradication therapies*

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SUMMARY

Background: There are no guidelines on second-line therapies for *Helicobacter pylori* eradication failures of omeprazole clarithromycin–amoxicillin triple therapy.

Aim: To compare the efficacy of two second-line therapies for persistent *H. pylori* infection.

Methods: Over a 6-year period, patients with persistent *H. pylori* infection following omeprazole clarithromycin–amoxicillin eradication therapy were randomized to receive omeprazole, 20 mg twice daily, bismuth, 120 mg four times daily, metronidazole, 500 mg twice daily, and either tetracycline, 500 mg four times daily, or clarithromycin, 500 mg twice daily, given for 7 days. Before therapy, patients underwent endoscopy with biopsies for histology, culture and antibiotic susceptibility tests. *H. pylori* infection was confirmed by histology.

Results: Of the 95 randomized patients, 88 (93%) completed the study. Age, sex, smoking, ulcer/non-ulcer dyspepsia ratio and antibiotic resistance were not significantly different between the treatment groups. On intention-to-treat analysis, eradication was achieved in 41 of the 49 patients (84%; 95% confidence interval, 70.4–92.7%) and 27 of the 46 patients (59%; 95% confidence interval, 43.3–73.0%) of the tetracycline- and clarithromycin-containing groups, respectively ($P = 0.007$). On multivariate regression analysis, the sensitivity of *H. pylori* to metronidazole had a likelihood ratio of 5.2 ($P = 0.022$), followed by the type of quadruple therapy (likelihood ratio, 4.4; $P = 0.036$).

Conclusions: Tetracycline-containing quadruple rescue therapy is highly effective in treating *H. pylori* eradication failures of the omeprazole amoxicillin–clarithromycin regimen.

INTRODUCTION

Omeprazole–clarithromycin–amoxicillin *Helicobacter pylori* eradication therapy given for 10 days (OCA-10) is highly effective,[1, 2] and is recommended by the Maastricht-2 2000 consensus report as first-line therapy.[3] Despite this, most studies have shown a 10–23% failure rate with this regimen.[3, 4] Patients with persistent *H. pylori* infection are at risk of peptic ulcer relapses and the development of complications of peptic ulcer disease. They therefore need further therapy to eradicate *H. pylori*.[5, 6]

Retreatment of *H. pylori* infection is often difficult in clinical practice.[7] This is because first-line treatment failures may lead to the selection of 'difficult to kill' bacterial strains and the development of secondary resistance to key antibiotics used as first-line treatment.[8, 9] It has been suggested that quadruple regimens may overcome the therapeutic difficulties arising when first-line proton pump inhibitor triple therapy fails.[10] Nevertheless, their efficacy in clinical practice has not yet been adequately evaluated.[11, 12]

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When selecting a retreatment regimen, the first-line treatment used and the microbial resistance to antibiotics should be taken into account.[10] The avoidance of antibiotics used in a previously failed eradication attempt is wise, but it has been shown that certain triple or quadruple combinations may exhibit some synergism, allowing resistance to the antibiotic components to be overcome.[13, 14] Therefore, the aim of the present randomized study was to evaluate the efficacy and tolerability of two quadruple regimens in patients with persistent *H. pylori* infection after a failed OCA-10 regimen. One quadruple regimen included omeprazole, bismuth, metronidazole and tetracycline (OBMT). Clarithromycin was substituted for tetracycline in the other regimen (OBMC). OBMT is a typical salvage therapy. OBMC has not been validated so far. It is a modification of OMC triple therapy with the addition of bismuth.

PATIENTS AND METHODS

Patients

The study was performed in two departments using OCA-10 as first-line therapy for patients with *H. pylori* infection over a 6-year period (1996–2001). Each patient who failed eradication with OCA-10 triple therapy was invited to be randomized into one of two quadruple *H. pylori* eradication therapies, one including tetracycline (OBMT) and the other clarithromycin (OBMC). Ninety-five (96%) of the 99 patients with persistent *H. pylori* infection accepted to participate in the study. They included 59 men and 36 women (median age, 45 years; range, 18–79 years). Patients were randomized according to a computer-generated randomization list. Before first-line therapy, 30 patients had duodenal ulcer and 65 had non-ulcer dyspepsia.

All patients gave written informed consent after full explanation by the investigator. The study protocol was approved by the Ethical Committee on Human Studies of the Department of Medicine, Medical School, Athens University. No patient had received other *H. pylori* eradication therapy, apart from the OCA-10 regimen, or antibiotics, bismuth, proton pump inhibitors or non-steroidal anti-inflammatory drugs during the month preceding the study. Pregnant or lactating women, patients who had undergone gastric surgery and those with severe chronic diseases were excluded from the study.

Diagnosis and treatment of persistent H. pylori *infection*

Following randomization into rescue therapy, each patient underwent a repeat upper gastrointestinal endoscopy with biopsies ($n = 6$) to confirm *H. pylori* infection and to investigate the sensitivity of *H. pylori* to antibiotics. Two antral and two corpus biopsy specimens were used for histology and one biopsy taken from the lesser and another from the greater curvature of the antrum were used for culture.

The second-line treatment regimens were given for 7 days. Patients and investigators were not blind to the treatments. OBMT rescue therapy included omeprazole, 20 mg twice daily, tripotassium dicitrato bismuthate, 120 mg four times daily, metronidazole, 500 mg twice daily, and tetracycline chlorhydrate, 500 mg four times daily. Four times dosing of tetracycline and bismuth were given, as initially published.[14] Clarithromycin, 500 mg twice daily, was used instead of tetracycline in the OBMC treatment arm. Endoscopy was repeated 4–6 weeks after second-line therapy to confirm *H. pylori* eradication. Four biopsies were taken (two from the lesser curvature of the antrum and two from the greater curvature of the corpus) and sent to the pathology department for histology. Modified Giemsa staining was used to detect *H. pylori* in biopsy specimens. The Warthin–Starry technique was employed for negative cases. A senior pathologist (C.S.), blind to randomization, evaluated all biopsy specimens. Treatment side-effects were investigated by interview.[15] Compliance with therapy was assessed by the number of tablets returned by each patient at final follow-up.

Susceptibility tests of H. pylori *isolates to antibiotics*

The *H. pylori* isolates were recovered from gastric biopsies and stored in brain heart infusion broth containing glycerol at −80 °C until testing. Minimal inhibitory concentrations of metronidazole, clarithromycin and tetracycline were determined by an agar dilution method, using Mueller Hinton agar supplemented with 7% horse blood and containing the antibiotics at the designated concentrations as previously described.[16] The minimal inhibitory concentration was taken as the lowest antibiotic concentration that inhibited visible growth after incubation at 37 °C in a microaerophilic atmosphere for 72 h. The breakpoints to define resistance were: > 2 mg/mL for clarithromycin, > 4 mg/mL for tetracycline and > 8 mg/mL for

metronidazole. The bacteriologist (A.M.) was blind to randomization and the results of histology.

Statistical analysis

All data were stored in a dBase software (Microsoft Access 2000, Microsoft Corp., USA). Analysis of the results was performed by statistical package SPSS 10.0.0 (SPSS Inc., Chicago, IL, USA). Data in the text and tables are presented as medians with ranges. Percentages are given with 95% confidence intervals (CI). Numerical data were analysed by either two-tailed *t*-test or the non-parametric Mann–Whitney *U*-test as appropriate. Qualitative data were evaluated by two-sided Pearson's chi-squared test or Fisher's exact test as appropriate, with both 'per protocol' and 'intention-to-treat' analysis. The former considers only the patients completing the study and the latter includes all patients abandoning the study as treatment failures.

Treatment outcome was assessed by intention-to-treat analysis. Assuming a difference of 20% in eradication efficacy between the two treatment groups to be clinically relevant, 46 patients should be included per treatment group, based on a two-sided test with a power of 80% and an alpha error of 5% (Statistical package GraphPad StatMate 1.01, San Diego, CA, USA).

Multivariate logistic regression analysis, with a forward stepwise selection criterion of < 0.05 for entering the model and a removal criterion of a likelihood ratio of > 0.1, was used to identify variables associated with success of treatment. The dependent variable was 'outcome' (success, 1; failure, 0) and the independent variables were sex (male, 1; female, 0), 'age', 'compliance' (percentage of tablets used by the patient), 'smoking' (yes, 1; no, 0), 'disease' (duodenal ulcer, 1; non-ulcer dyspepsia, 0) and 'therapeutic regime' (OBMT, 1; OBMC, 0). A P value of less than 0.05 was regarded as significant.

RESULTS

Over a 6-year period (1996–2001), 566 patients had follow-up endoscopy in our departments 4–8 weeks after *H. pylori* eradication therapy with OCA-10. One hundred and three (18.2%) had persistent *H. pylori* infection (Figure 1). Two patients did not meet the inclusion criteria and six refused to participate in the study. Therefore, 95 (93%) patients accepted randomization into second-line *H. pylori* eradication therapy with either OBMT or OBMC. The distribution of the clinical and demographic characteristics studied was not significantly different between treatment groups (Table 1). Seven patients (8%), three in the OBMT treatment group and four in the OBMC treatment group, were lost to follow-up. Therefore, 88 patients completed the study, including 46 of the 49 in the OBMT group and 42 of the 46 in the OBMC group.

Eradication of H. pylori

Intention-to-treat analysis showed that the eradication of *H. pylori* was successful in 41 of the 49 patients treated with the OBMT regimen (83.7%; 95% CI, 70.4–92.7%) and in 27 of the 46 patients treated with the OBMC regimen (58.7%; 95% CI, 43.3–73.0%) ($\chi^2 = 7.2$, $P = 0.007$). The respective values for the per protocol analysis were 41/46 (89.1%; 95% CI, 76.4–96.4%) and 27/42 (64.3%; 95% CI, 48.0–78.4%) patients ($\chi^2 = 7.7$, $P = 0.005$).

Multivariate regression analysis showed that therapy with OBMT was the single variable significantly related to successful *H. pylori* eradication (odds ratio, 4.5; 95% CI, 1.5–14.0; $P = 0.008$). Sex, age, smoking status, disease (duodenal ulcer/non-ulcer dyspepsia) and compliance with therapy were rejected by the model.

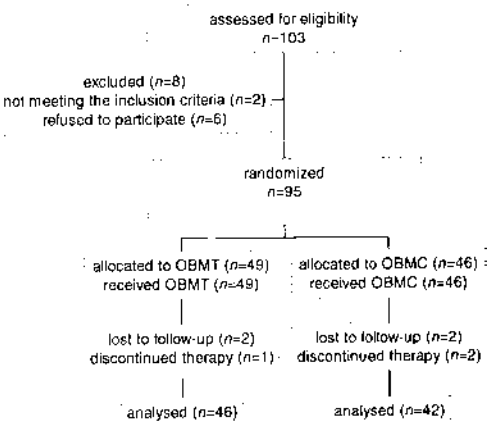

Figure 1. Flow diagram of the progress of patients through the phases of randomization.

Variable	OBMT (n = 49)	OBMC (n = 46)	P value
Age (years) (median with range)	43 (18–78)	44 (19–78)	0.97§
Sex (male/female)	31/18	28/18	0.81‡
Smoking (% smokers)	50.0	39.5	0.24‡
Duodenal ulcer/non-ulcer dyspepsia*	13/36	17/29	0.27‡
Lost to follow-up	2	2	
Discontinued treatment	1	2	
Tablets used (%) (median with range)	100 (89–100)	100 (86–100)	0.66†

Table 1. Demographic characteristics of patients in the tetracycline-containing quadruple therapy (OBMT) and clarithromycin-containing quadruple therapy (OBMC) groups

OBMT, omeprazole, bismuth, metronidazole, tetracycline; OBMC, omeprazole, bismuth, metronidazole, clarithromycin.
*Diagnosis before first-line therapy.
†Mann–Whitney U-test.
‡Two-sided Pearson's chi-squared test.
§Two-tailed t-test.

Effect of microbial resistance on eradication therapy

Antibiotic resistance patterns were determined in isolates obtained at endoscopy from 73 of the 95 patients (77%; 95% CI, 67.1–84.9%) who had an initially failed eradication attempt. The prevalence of metronidazole and clarithromycin resistance was 39.7% and 24.7%, respectively. Dual resistance to metronidazole and clarithromycin was recorded in 10 out of 73 (13.7%) *H. pylori* strains (Table 2). All isolated *H. pylori* strains were susceptible to tetracycline. Six of these 73 antibiotic sensitivity tests corresponded to patients who had either discontinued therapy (n = 2) or were lost to follow-up (n = 4). Therefore, 67 sensitivity tests were included in the analysis.

Figures 2(a) and 3(a) show that, before second-line therapy, metronidazole- and clarithromycin-sensitive ($\chi^2 < 0.2$, $P > 0.32$) (Figure 2a) and -resistant ($\chi^2 < 0.72$, $P > 0.4$) (Figure 3a) strains were equally allocated in the OBMT and OBMC treatment groups. The comparison of *H. pylori* eradication rates between OBMT and OBMC groups showed that both regimens were equally effective in metronidazole-sensitive (Fisher's exact test, $P = 0.3$) and clarithromycin-sensitive (Fisher's exact test, $P = 0.7$) strains (Figure 2b), as well as in metronidazole-resistant strains (Fisher's exact test, $P = 0.2$) (Figure 3b). However, the OBMT regimen was more effective than the OBMC regimen in clarithromycin-resistant strains (Fisher's exact test, $P = 0.007$) (Figure 3b).

However, when we included metronidazole and clarithromycin resistance (0, sensitive; 1, resistant) in our multivariate regression model, it was shown that metronidazole sensitivity played a significant role (odds ratio, 4.15; 95% CI, 1.13–15.26) in the *H. pylori* eradication rates. Sensitivity to metronidazole entered the model first (likelihood ratio, 5.22; $P = 0.022$), followed by the type of quadruple therapy (likelihood ratio, 4.42; $P = 0.036$), while clarithromycin was rejected by the model.

Side-effects and compliance with therapy

Twenty-five (54%) patients in the OBMT group and 29 (69%) patients in the OBMC group (χ^2 0.1, P 0.65) experienced mild side-effects, such as fatigue, taste disturbance, nausea, loose stools or epigastric pain. Four (8.7%) patients treated with OBMT and 12 (28.6%) patients treated with OBMC ($\chi^2 = 2.8$, $P = 0.07$) reported more severe side-effects, such as

	Total cultures (n = 67)		OBMC (n = 36)		OBMT (n = 37)	
Sensitivity	Number	%	Number	%	Number	%
MSCS	36	49.32	16	44.44	20	54.05
MSCR	8	10.96	3	8.33	5	13.51
MRCS	19	26.03	11	30.56	8	21.62
MRCR	10	13.7	6	16.67	4	10.81

Table 2. Patterns of antibiotic sensitivity tests in the tetracycline-containing quadruple therapy (OBMT) and clarithromycin-containing quadruple therapy (OBMC) groups. Sensitive and resistant *Helicobacter pylori* strains were not significantly different in the two treatment groups ($\chi^2 = 1.8$, DF = 3, $P = 0.62$)

OBMT, omeprazole, bismuth, metronidazole, tetracycline; OBMC, omeprazole, bismuth, metronidazole, clarithromycin; C, clarithromycin; M, metronidazole; S, sensitive; R, resistant.

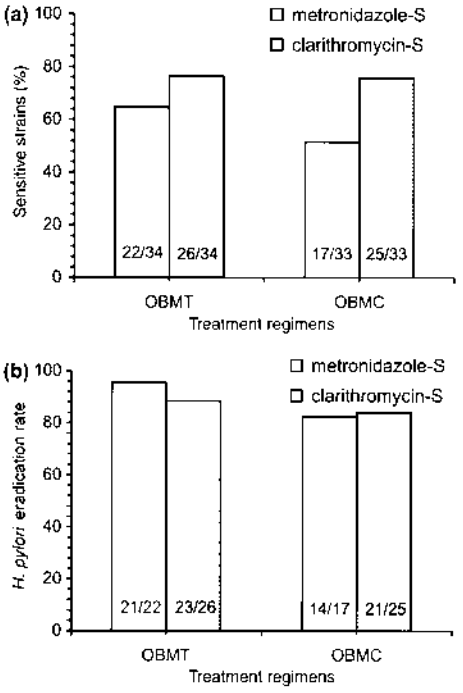

Figure 2. (a) Metronidazole- and clarithromycin-sensitive *Helicobacter pylori* strains were equally allocated in the tetracycline-containing quadruple therapy (OBMT) and clarithromycin-containing quadruple therapy (OBMC) groups ($\chi^2 < 0.2$, $P > 0.3$). (b) *H. pylori* eradication rates were not significantly different between OBMT and OBMC treatment groups for metronidazole- and clarithromycin sensitive *H. pylori* strains ($P > 0.3$).

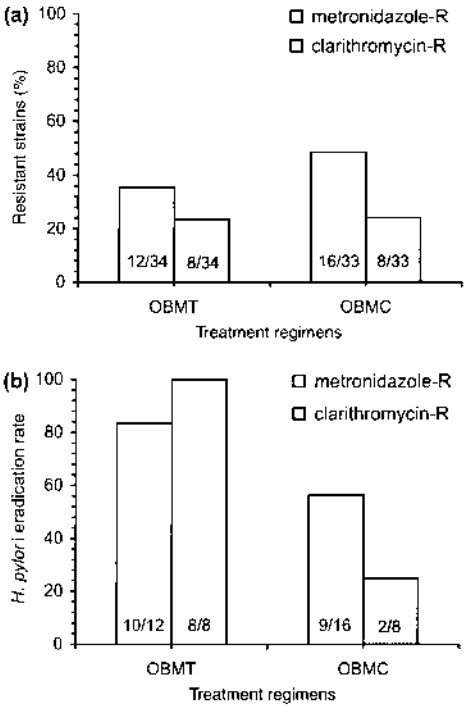

Figure 3. (a) Metronidazole- and clarithromycin-resistant *Helicobacter pylori* strains were equally allocated in the tetracycline containing quadruple therapy (OBMT) and clarithromycin containing quadruple therapy (OBMC) groups ($\chi^2 < 0.72$, $P > 0.4$). (b) OBMT and OBMC were equally effective in eradicating metronidazole-resistant strains ($P = 0.2$). However, OBMT was more effective than OBMC in eradicating clarithromycin-resistant strains ($P = 0.007$).

oral candidiasis, diarrhoea or vomiting. These treatment side-effects appeared during the last 3 days of the 7-day period. Only three patients discontinued therapy (Table 1, Figure 1). All patients that returned to follow-up were compliant with the treatment and had taken > 85% of the assigned tablets. Compliance with therapy, i.e. the percentage of tablets taken, was not significantly different between the OBMT and OBMC treatment groups (100, 89–100% vs. 100, 86–100%, respectively; $Z = -0.437$; $P = 0.66$).

DISCUSSION

In this prospective, randomized study, we compared the efficacy of two quadruple, second-line, *H. pylori* eradication therapies. One treatment arm included omeprazole, bismuth, metronidazole and tetracycline (OBMT) and in the other tetracycline was replaced by clarithromycin (OBMC). We also evaluated the impact of bacterial resistance on the *H. pylori* eradication rates.

Our patients had initially been treated with the OCA-10 regimen. This is a highly effective therapy in treating *H. pylori* infection.[1] In our departments, OCA-10 had a failure rate of 18.2%, which agrees with the results of published reports.[2–4] First-line treatment failure may lead to the development of acquired bacterial resistance to key antibiotics.[1,7] Following the OCA-10 regimen, 25% of the *H. pylori* strains isolated from our patients were resistant to clarithromycin and 40% to metronidazole. Therefore, the selection of an appropriate second-line therapy is challenging for the clinician.

Our study showed that quadruple therapy with OBMT was more effective than that with OBMC in treating *H. pylori* eradication failures following OCA-10 first-line eradication therapy. According to the Maastricht-2 2000 consensus report, first-line regimens which do not include metronidazole may lead to better results with a quadruple second-line regimen including this antibiotic.[3] Preliminary results have shown that, although the OBMT regimen has been successfully used after failure of clarithromycin-containing first-line therapies, eradication rates are usually low after first-line triple therapies including metronidazole because of secondary metronidazole resistance.[18, 19] Although recent data have suggested that certain combinations of metronidazole with clarithromycin and acid suppressors or bismuth compounds may exhibit synergistic effects, overcoming bacterial resistance,[20, 21] this was not confirmed by our study.

The *H. pylori* eradication rate (84%) obtained in our study using the OBMT quadruple regimen was lower than the eradication rates reported by other investigators (93%) using OBMT as first-line therapy.[22] Several studies have investigated OBMT as second-line *H. pylori* therapy, with a wide variation of *H. pylori* eradication rates (57–95%). The order of success seems to favour studies using higher doses of metronidazole.[11, 12, 23] The overall prevalence of metronidazole-resistant strains in our study was close to 49%, which has been reported for our country.[24] The tetracycline-containing quadruple regimen eradicated nearly all the metronidazole-sensitive (95%) and most of the metronidazole-resistant (83%) *H. pylori* strains. These figures are compatible with published reports.[25]

Our results showed that metronidazole resistance significantly affected the overall outcome. However, when comparing *H. pylori* eradication rates between second-line therapies, where *H. pylori* metronidazole-resistant strains were not differently allocated in each treatment group, the type of regimen was a major factor predicting treatment outcome. This could be related to the efficacy of the other components of each regimen, i.e. omeprazole, bismuth, clarithromycin or tetracycline.[26] The substitution of tetracycline by clarithromycin reduced the efficacy of second-line therapy, probably because of secondary resistance of *H. pylori* strains to clarithromycin.

Our study may be criticised because, ideally, two methods should be used to confirm *H. pylori* infection. Following *H. pylori* quadruple eradication therapy, we used the histology of four biopsies which were taken from the gastric antrum and body; this has been shown to reduce the sampling error.[27] An unequivocal single gold standard does not exist for the confirmation of *H. pylori* infection. However, histology with Warthin–Starry stain gives the best sensitivity (93%) and specificity (99%)[28] when compared with urea breath test.[29]

Overall, the side-effects were mild and did not interfere with compliance in our study. Other studies using higher metronidazole doses have reported an increased rate of side-effects with quadruple therapy.[30, 31] Only three patients discontinued treatment in our study.

OBMT, besides being much cheaper (37.1 euro) than OBMC (75.6 euro), was a more effective second-line therapy for treatment failures of OCA-10. When clarithromycin fails as first-line therapy, avoidance of its use in salvage therapies is wise,[32] even if bismuth is added.

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ORIGINAL INVESTIGATION

Eradication of *Helicobacter pylori* May Be Beneficial in the Management of Chronic Open-Angle Glaucoma

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Background: We have documented a high prevalence of *Helicobacter pylori* infection in patients with glaucoma.

Objective: To evaluate the effect of *H pylori* eradication on the 2 most commonly used glaucoma parameters: intraocular pressure and visual field.

Methods: A total of 41 patients with glaucoma and 30 age-matched anemic controls underwent upper gastrointestinal endoscopies and gastric mucosal biopsies to detect the presence of *H pylori* infection by histologic analysis and rapid urease test (CLOtest; Delta West, Draper, Utah). Saliva samples were also tested by CLOtest. Serum anti–*H pylori*–specific IgG was analyzed by enzyme-linked immunosorbent assay. *Helicobacter pylori*–positive patients received a triple eradication regimen (omeprazole, clarithromycin, and amoxicillin treatment), and all patients were observed for 2 years while remaining under the same antiglaucoma therapy.

Results: *Helicobacter pylori* was detected in 88% of glaucoma cases and in 47% of controls ($P<.001$). *Helicobacter pylori* eradication was successful in 83% of treated patients. At the 2-year clinical end point, glaucoma parameters (mean intraocular pressure and mean visual field parameters) were improved in the subgroup of patients where *H pylori* eradication was successful ($P<.001$ for intraocular pressure; $P\leq.01$ for visual field parameters), but not in the other patients.

Conclusion: *Helicobacter pylori* eradication may positively influence glaucoma parameters, suggesting a possible causal link between *H pylori* and glaucoma.

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GLAUCOMA COMPRISES a large group of age-related disorders characterized by widely diverse clinical and morphological manifestations.[1] The common characteristic of all forms of glaucoma is progressive optic neuropathy, which derives from a multitude of risk factors. Recently, it has become clear in animal models of glaucoma that retinal ganglion cells die via apoptosis.[2] Currently, the best-known causative risk factor is raised intraocular pressure.[1] Other less well-understood risk factors include ischemia, vascular dysregulation, and low systemic blood pressure.[2,3] These factors induce ocular blood flow deficits that either cause direct damage or increase the susceptibility of the neural tissue in the optic nerve. Recent evidence suggests that glaucomatous optic neuropathy may be associated with changes in endothelium-dependent vascular regulation, impaired ocular blood flow, and cytokines.[3]

Glaucoma is currently the second most common cause of blindness in the world.[3,4] Although glaucoma-related loss of vision cannot be reversed in adult patients, blindness can be prevented in nearly all cases with early detection and proper therapy.[4] Nevertheless, the only available therapeutic intervention in glaucoma remains the reduction of intraocular pressure by topical drugs, laser treatment, or surgery,[4] and this approach fails to address the events leading to the elevated intraocular pressure. A far more effective goal in the treatment of glaucoma might be to address the various risk factors leading to glaucomatous neuropathy. However, appropriate management of glaucoma requires a better understanding of the pathogenetic mechanisms involved.

Helicobacter pylori is a curved spiral gram-negative bacterium that colonizes the gastric mucosa of most humans worldwide, mainly affecting older adults in the developed world, including Greece.[5,6] It is associated with various upper gastrointestinal (GI) diseases[5,7] and has also been implicated in a variety of extradigestive vascular conditions including ischemic heart disease,[8] ischemic cerebrovascular disorders,[9] and functional vascular disorders caused by vascular dysregulation (eg,

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PATIENTS AND METHODS

PATIENTS

This was a 2-part study. Part 1 was designed to evaluate the prevalence of *H pylori* infection in chronic OAG.[12] Forty-one patients with documented chronic OAG and 30 age-matched anemic controls were included in this part of the study. The control subjects were undergoing upper and lower GI endoscopy to investigate mild iron deficiency anemia, but their endoscopy findings were normal. After a detailed history was taken from all patients and their first-degree relatives, 26 (63%) patients with glaucoma were found to have intermittent symptoms of dyspepsia (defined as pain, discomfort, or other symptoms believed to originate from the upper GI tract), heartburn, or a history of upper GI tract bleeding. All patients with glaucoma (36 *H pylori*–infected patients who received the *H pylori* eradication regimen and 5 *H pylori*–negative patients who did not receive eradication therapy) were subsequently observed in the second part of the study, which evaluated the effect of administration of *H pylori* eradication regimen on intraocular pressure and visual field parameters over a 2-year follow-up period.

Patients were enrolled in the study if they met the following criteria: (1) an untreated intraocular pressure of 21 mm Hg or higher; (2) typical glaucomatous optic nerve head changes (including saucerization, rim thinning or notching in the inferior or superior temporal area of the optic nerve head, or total glaucomatous cupping)[12]; and/or (3) typical glaucomatous visual field loss (including a paracentral, arcuate, or Seidel scotoma or a nasal step).[12] Seven of the patients enrolled were legally blind in the contralateral eye due to end-stage glaucoma. To reduce the possible bias of age, all participants were older than 45 and younger than 70 years. Exclusion criteria included all eye diseases other than glaucoma, diabetes mellitus, and a myopic refractive error exceeding −8 diopters. In addition, patients were excluded if they had taken H_2-receptor antagonists, proton pump inhibitors, antibiotics, bismuth compounds, or nonsteroidal anti-inflammatory drugs in the preceding 4 weeks (excluding low doses of aspirin [ie, 80 mg 2-3 times daily]). Patients were also excluded if they had undergone previous gastric surgery; were receiving anticoagulant therapy; were alcohol abusers; had allergy to penicillin or macrolides, had gastric cancer or other neoplasms; or had severe cardiac, pulmonary, kidney, or liver disease.

All patients signed a consent form prior to enrollment, and the study protocol was approved by the local ethics committee. The intraocular pressure was measured with a calibrated Goldmann applanation tonometer by the same ophthalmologist. The visual field parameters were measured with the G1 Octopus program (Octopus 300EZ G1; Interzeag AG, Zurich, Switzerland) by the same perimetrist. The ophthalmologist and the perimetrist were masked to the *H pylori* status of the patient. All patients had prior experience with automated perimetry. The parameters assessed included corrected loss variance (CLV), mean defect (MD), and short-term fluctuation (SF) of both eyes. The G1 program evaluates the threshold at 59 points within the central 30° of the visual field. Normal intraocular pressure generally varies between 12 and 20 mm Hg.[12] The normal spread of the visual field parameters are SF, 0 to 2 dB; CLV, 0 to 4 dB; and MD −2 to +2 dB.[12] The SF describes the intratest variation of the retinal sensitivity and may be affected by test-retest experience. The CLV shows the nonuniform variation from the expected hill of vision minus the effect of the SF. The MD represents the average decibel defect per location from the expected norm and may be influenced by refraction, the pupil size, and media opacities. It should be emphasized that no definite rules exist to indicate whether a patient's condition is progressing or is stable.

Only topical glaucoma treatment was used in the 41 patients included in this study. The details of their treatment have been reported previously.[12] None of the patients in this study received oral drugs that could influence intraocular pressure (eg, carbonic anhydrase inhibitors). All patients with glaucoma received the same topical eye regimen during the 2 year follow-up period of this study.

The control subjects had been recently diagnosed as having mild iron-deficiency anemia. The diagnosis of anemia was based on history and the GI investigation, and none of the subjects had received any treatment (eg, ferrous sulfate) prior to the diagnosis. The details of the their blood profile and the causes of iron-deficiency anemia have been reported previously.[12]

All 71 participants (41 patients with glaucoma, 30 anemic controls) underwent elective upper GI endoscopy combined with diagnostic biopsies at baseline. In addition, all patients with glaucoma underwent the same endoscopic procedure 3 months after *H pylori*–eradication treatment. Patients with peptic ulcer disease who agreed to undergo repeated elective follow-up endoscopies with biopsies were followed up with elective endoscopic procedures at 6 and 12 months to rule out *H pylori* reinfection.

STUDY DESIGN

Subjects reported for the procedures at 9 AM after a 12-hour fast. Intravenous sedation was given, and standard upper GI endoscopy was performed with a forward viewing videoscope (Olympus CF 0197; Opto-Electronics Co Ltd, Tokyo, Japan) for evaluation of any macroscopic abnormalities. Simultaneously, 3 biopsy specimens were obtained from the antral region within 2 cm of the pyloric

Raynaud phenomenon and migraine)[10] and with some autoimmune conditions such as Sjögren syndrome.[11]

Until now, no attempt has been made to investigate the prevalence of *H pylori* in glaucoma patients. Only recently, Kountouras and associates[12] reported a higher prevalence of *H pylori* in Greek patients with open-angle glaucoma (OAG) than in age-matched controls, suggesting for the first time an association between *H pylori* infection and glaucoma in this ethnic cohort. Demonstrating the association of *H pylori* and glaucoma and proving the benefit of eradicating *H pylori* in the clinical course of the disease may have a major impact on treatment. Nevertheless, before antibiotic therapy for *H pylori* infection becomes an established step in the management of chronic OAG, sufficient evidence must be provided that glaucoma

ring and 3 from the corpus. A biopsy specimen from each site was used for rapid urease slide testing for *H pylori* infection (CLOtest; Delta West, Draper, Utah), and the other 2 biopsy specimens were placed in 10% formalin and submitted for histologic examination. Before endoscopy, venous blood was drawn from each patient for serologic testing for *H pylori* IgG antibodies. Serum samples were stored at −20°C for analysis within 20 to 25 days for *H pylori* IgG antibodies by using an enzyme-linked immunosorbent assay technique (Elias, Osceola, Wis). Simultaneously, saliva samples were also collected in sterile tubes for rapid urease activity testing. To prevent contamination of specimens from different sites, biopsy specimens from each site were taken with a fresh pair of sterile forceps. The forceps were wiped with alcohol on withdrawal from the endoscope to remove any organism that might have been present in the biopsy channel. Endoscopes were sterilized between procedures according to standard guidelines.[14]

BIOPSY AND SALIVA UREASE TESTS

Each biopsy specimen and saliva sample was placed in a tube containing 0.5 mL of 10% urea in deionized water to which had been added 2 drops of 1% phenol red as a pH indicator (CLOtest). The biopsy specimen test was read at 5 minutes, 1 hour, 3 hours, and 24 hours and was considered positive if the indicator changed from yellow to red at any time. The saliva sample test was read at 5 minutes, 1 hour, and 3 hours.[12,15]

HISTOPATHOLOGIC ANALYSIS

All specimens were stained with hematoxylin-eosin and Crezyl fast violet and/or Giemsa stain (for detection of *H pylori* organisms). The presence of gastritis was classified in accordance with the Sydney System and included assessment of atrophy grade, chronicity, activity, and intestinal metaplasia on a scale of 0 (absent) to 3 (high), as previously reported.[12] In particular, intestinal metaplasia was evaluated with Alcian blue stain in addition to hematoxylin-eosin. The same experienced pathologist, masked to the other determinants of *H pylori* status and to the patients' group, assessed all specimens.

H PYLORI SEROLOGIC TESTING

Helicobacter pylori serologic status was determined by using a commercial enzyme linked immunosorbent assay kit (Elias). The manufacturer's recommended cutoff value of 10 U/mL for *H pylori* IgG, validated in our laboratories, was used to define patient serologic findings as positive or negative.

The diagnosis of *H pylori* infection was based on histologic findings. In particular, *H pylori* infection was assumed when active gastritis with bacteria of typical shape was found histologically. Other parameters used were positive CLOtest findings (biopsy specimens and saliva) and *H pylori* IgG serologic results.

TREATMENT OF *H PYLORI* INFECTION

To treat *H pylori* infection, a 1-week course of omeprazole (20 mg twice a day), clarithromycin (500 mg twice a day), and amoxycillin (1 g twice a day) was given followed by 20 mg of omeprazole daily for 1 month. The patients completed a questionnaire to record the adverse effects occurring during treatment and their compliance with the therapy. The adverse effects were recorded as absent, mild, moderate, or severe. Compliance was evaluated by counting tablets and capsules after therapy: good compliance was ensured when the patient had taken more than 90% of the tablets and capsules.

FOLLOW-UP SCHEME

Success of the *H pylori* eradication regimen was evaluated by control endoscopy at least 8 weeks after cessation of therapy, and we considered a patient to be *H pylori* negative if both histologic and the rapid urease test results were negative. Exclusion of *H pylori* reinfection was determined in patients with peptic ulcer who underwent repeated elective follow-up endoscopies with biopsies within 1 year. Glaucoma parameters were prospectively recorded at baseline and after 1 and 2 years of follow-up with the same topical medication regimen. The ophthalmologists treating the patients in this study were masked to the *H pylori* status of these patients.

The follow-up study population was classified into 3 glaucoma groups: patients for whom *H pylori* treatment was successful (group A); those for whom eradication of *H pylori* had failed (group B); and those who were *H pylori* negative at baseline (group C).

STATISTICAL ANALYSIS

The prevalence of *H pylori* and respective 95% confidence intervals (CIs) were calculated at 3 months after eradication treatment. For the patients' age (years), the Mann-Whitney *U* test was used, whereas for sex, the Fisher exact test was applied. The latter test was also used to assess the progress of the various upper GI endoscopic and histologic findings 3 months after treatment. An unpaired *t* test was applied to compare the glaucoma parameters of groups A and B with group C. For all glaucoma parameters (intraocular pressure and visual field data [CLV, MD, and SF]), comparisons were made between baseline and after 1 and 2 years of follow-up with the paired *t* test and 95% CIs of the difference. The paired *t* test was also applied to compare the *H pylori* serologic status at baseline with that at 3 months after treatment. Significance was set at $P<.05$.

parameters are positively influenced by the eradication of *H pylori* infection.

The objective of the present study is to evaluate the effect of *H pylori* eradication on 2 well-established clinical parameters in glaucoma: intraocular pressure and visual field indices.[13] We have therefore designed methods to confirm and quantify our hypothesis that *H pylori* eradication therapy has a beneficial effect on these 2 glaucoma parameters in *H pylori*–positive patients with glaucoma.

RESULTS

The patients with glaucoma had a higher prevalence of *H pylori* infection than controls, as verified by the his-

Table 1. *Helicobacter pylori* Positivity in Patients With Glaucoma and Controls*

Characteristic	Glaucoma Patients (n = 41)	Controls (n = 30)	Odds Ratio (95% CI)	P Value
Mean ± SD age (range), y	61.4 ± 7.9 (45-70)	62.2 ± 8.6 (44-70)	...	.27
No. of men/No. of women	20/21	14/16	...	.53
Positive urease test results				
Saliva	17 (42)	9 (30)	...	.23
Gastric mucosa	30 (73)	14 (47)	3.11 (1.13 to 8.59)	.02
Anti–*H pylori* IgG >10 U/mL	28 (68)	9 (30)	5.02 (1.81 to 13.91)	.002
Histologically confirmed presence of *H pylori*	36 (88)	14 (47)	8.23 (2.35 to 28.83)	<.001

*Unless otherwise indicated, data are number (percentage) of patients. CI indicates confidence interval; ellipses, not applicable.

Table 2. Endoscopic and Histologic Characteristics of the 3 Groups of Glaucoma Patients at Baseline and 3 Months After Treatment*

	Group A (n = 30)			Group B (n = 6)			Group C (n = 5)		
Characteristic	Baseline	3 mo	P Value	Baseline	3 mo	P Value	Baseline	3 mo	P Value
Esophagitis	5	2	.24	0	0	>.99	3	1	.26
Sydney System gastritis rating	30	30	>.99	6	6	>.99	5	5	>.99
0	0	7	.005	0	1	.50	2	3	.50
1	13	19	.10	1	4	.10	3	2	.50
2	12	4	.02	4	1	.10	0	0	>.99
3	5	0	.03	1	0	.50	0	0	>.99
Duodenitis	11	6	.13	4	2	.30	3	2	.50
Lower esophageal sphincter inefficiency	14	14	.6	0	0	>.99	3	3	.74
Hiatal hernia	2	2	.7	1	1	.77	1	1	.78
Peptic ulcer	5	0	.03	2	0	.23	0	0	>.99

*Group A includes only patients in whom *Helicobacter pylori* was successfully eradicated; group B, patients in whom the *H pylori* eradication regimen was unsuccessful; and group C, *H pylori*–negative patients at baseline; a indicates the number of patients treated.

tologically confirmed presence of *H pylori* in 36 (88%) of 41 glaucoma cases, including 6 patients who tested negative in the gastric mucosa urease test, and in 14 (47%) of the 30 control subjects (χ^2 = 14.075, P<.001; **Table 1**). The odds ratio for the association of *H pylori* with OAG was 8.23 (95% CI, 2.35-28.83). The mean serum IgG anti–*H pylori* level was also significantly higher in patients with glaucoma (35.6 ± 31.1 U/mL) than in controls (17.03 ± 18.1 U/mL; P = .002). In patients with glaucoma and controls, the diagnosis of esophagitis, gastritis, duodenitis, and/or peptic ulcer was made during endoscopy and confirmed histologically (**Table 2**).

OUTCOME OF *H PYLORI* ERADICATION THERAPY

Helicobacter pylori eradication therapy was successful in 30 (83%) of the 36 patients who were positive for *H pylori* at baseline (group A). Treatment was unsuccessful in the remaining 6 patients (group B). All patients were compliant with their therapy as determined by the number of tablets and capsules remaining after therapy. Adverse effects were generally mild and included mild abdominal pain, occasional nausea or vomiting, diarrhea, stomatitis, and headache. None of the patients discontinued their therapy because of these mild adverse effects.

When compared with baseline values (42.6 ± 31.0 U/mL), the mean serum IgG anti–*H pylori* level was significantly reduced in group A patients at 3-month follow-up (19.6 ± 14.3 U/mL) (P<.001). In group B patients, this parameter had increased at 3-month follow-up (28.5 ± 21.6 U/mL at 3 months vs 25.4 ± 19.4 U/mL baseline; P = .04). In group C patients, who did not receive *H pylori* eradication therapy, both mean serum IgG anti–*H pylori* values were below positive-limit levels (7.2 ± 1.6 U/mL at 3 months vs 6.0 ± 1.9 U/mL at baseline; P = .03).

All 5 group A patients with ulcers (2 duodenal and 3 gastric) were endoscopically confirmed to have healed at 3-month follow-up (P = .03; Table 2). In group B, endoscopic parameters and chronic gastritis activity did not show statistical differences between baseline readings and the values at 3 months, although ulcer healing did occur in 2 duodenal ulcers in this group. All 5 patients in group A with peptic ulcer disease who were reexamined 1 year after undergoing the eradication regimen remained free of the infection.

At baseline, comparisons for endoscopic evidence of esophagitis and duodenitis did not reveal significant differences between patients with glaucoma and controls. Patients with glaucoma more often than controls exhibited histologically confirmed antral gastritis (38/41 vs 17/30; P<.001) and peptic ulcer disease (7/41 vs 0/30; P = .01).

Table 3. Comparison of Mean Intraocular Pressure and Visual Field Parameters for All Glaucoma Cases at Baseline and After 1 and 2 Years of Follow-up*

Patient Group†	Mean ± SD Measurement Value			Change From Baseline at 1 Year		Change From Baseline at 2 Years	
	Baseline	1-Year	2-Year	MDM (95% CI)	*P* Value	MDM (95% CI)	*P* Value
Intraocular Pressure, mm Hg							
A (n = 56)	18.91 ± 3.6	17.84 ± 2.3	17.70 ± 2.0	1.07 (0.26 to 1.88)	.005	1.21 (0.49 to 1.94)	<.001
B (n = 9)	19.00 ± 4.3	18.67 ± 2.6	18.22 ± 1.8	0.33 (−2.13 to 2.79)	.38	0.78 (−2.01 to 3.57)	.27
C (n = 9)	18.56 ± 2.9	18.33 ± 3.0	18.89 ± 2.9	0.22 (−1.26 to 1.70)	.37	0.33 (−2.1 to 1.43)	.34
Short-term Fluctuation							
A	2.44 ± 0.8	2.16 ± 0.9	1.99 ± 0.6	0.29 (0.03 to 0.54)	.02	0.46 (0.22 to 0.69)	<.001
B	1.94 ± 0.4	2.43 ± 0.6	3.13 ± 1.0	−0.49 (−1.15 to 0.17)	.06	−1.19 (−2.01 to −0.37)	.005
C	2.01 ± 1.0	2.22 ± 0.8	2.09 ± 1.1	−0.21 (0.56 to 0.14)	.10	−0.08 (−0.59 to 0.43)	.37
Corrected Loss Variance							
A	12.23 ± 15.8	9.93 ± 14.4	8.63 ± 12.3	2.30 (0.55 to 4.05)	.005	3.60 (1.03 to 6.18)	.003
B	16.53 ± 25.4	16.91 ± 24.9	22.70 ± 24.3	−0.38 (−2.29 to 1.53)	.33	−6.17 (−11.63 to −0.70)	.02
C	2.77 ± 3.3	3.03 ± 3.0	1.89 ± 1.0	−0.27 (−3.31 to 2.77)	.42	0.88 (−1.41 to 3.16)	.20
Mean Defect							
A	3.43 ± 5.2	3.08 ± 4.6	2.63 ± 4.6	0.35 (−0.66 to 1.35)	.25	0.80 (0.10 to 1.49)	.01
B	5.74 ± 5.8	4.82 ± 4.9	4.62 ± 4.8	0.92 (−2.41 to 4.25)	.27	1.12 (−0.32 to 2.57)	.06
C	0.56 ± 1.4	0.38 ± 1.1	0.8 ± 1.1	0.18 (−1.06 to 1.42)	.37	−0.24 (−1.00 to 0.51)	.24

*Unless otherwise indicated, all data are decibels (dB). MDM indicates mean difference of the means; CI, confidence interval.
†Group A includes only patients in whom *Helicobacter pylori* was successfully eradicated; group B, patients in whom the *H pylori* eradication regimen was unsuccessful; and group C, *H pylori*–negative patients at baseline; n indicates the number of eyes treated.

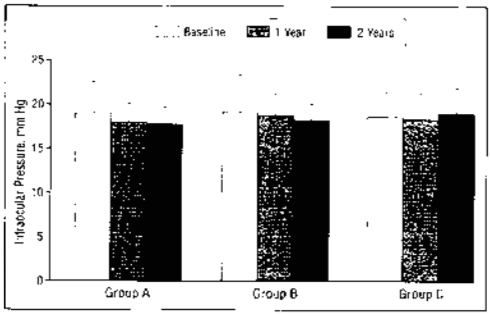

Figure 1. Mean intraocular pressure at baseline and 1 and 2 years after treatment in patients for whom *Helicobacter pylori* treatment was successful (group A); those for whom eradication of *H pylori* had failed (group B); and those who were *H pylori* negative at baseline (group C). Error bars indicate SD.

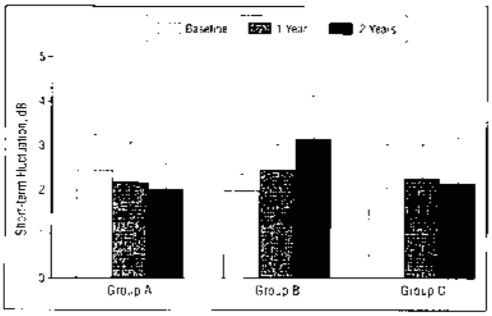

Figure 2. Mean short-term fluctuation at baseline and 1 and 2 years after treatment in patients for whom *Helicobacter pylori* treatment was successful (group A); those for whom eradication of *H pylori* had failed (group B); and those who were *H pylori* negative at baseline (group C). Error bars indicate SD.

OUTCOME OF GLAUCOMA PARAMETERS

Baseline visual field indices including MD and CLV were significantly lower in group C than in groups A and B ($P = .04$ for both), whereas no significant difference was observed in short-term fluctuation between these groups. **Table 3** and **Figures 1**, **2**, **3**, and **4** detail and illustrate the glaucoma parameters in all glaucomatous eyes (n = 74) of the 41 patients at baseline and 1 and 2 years after treatment. At the treatment end points selected in the present study (1 and 2 years), a significant improvement was found for all glaucoma parameters in group A compared with baseline readings except for MD at 1 year, the change for which did not reach statistical significance ($P = .25$). In contrast, glaucoma parameters did not differ or slightly deteriorated statistically from baseline to 1- and 2-year follow-up values in groups B and C.

COMMENT

Glaucomas comprise a group of age-related eye diseases that share common clinical and morphological features. These include an intraocular pressure too high for the health of the eye; progressive, insidious damage to the optic nerve; and visual field loss. It is estimated that more than 3 million Americans and more than 67 million people worldwide have glaucoma. Thus, glaucoma is currently the second leading cause of blindness in the Western world.[1-3]

Much remains to be learned about glaucoma, not only at the level of pathogenesis but also with regard to its systemic associations and influences. Among the key events in glaucoma are increased outflow resistance at the level of the trabecular meshwork, increased intraocular pressure, and a characteristic optic neuropathy.

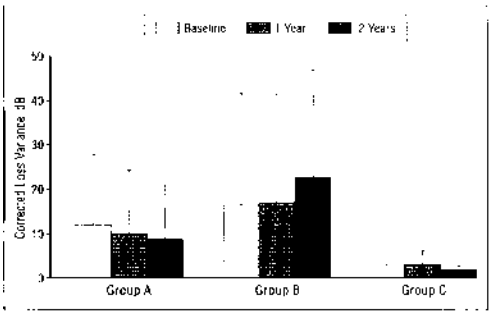

Figure 3. Mean corrected loss variance at baseline and 1 and 2 years after treatment in patients for whom *Helicobacter pylori* treatment was successful (group A); those for whom eradication of *H pylori* had failed (group B); and those who were *H pylori* negative at baseline (group C). Error bars indicate SD.

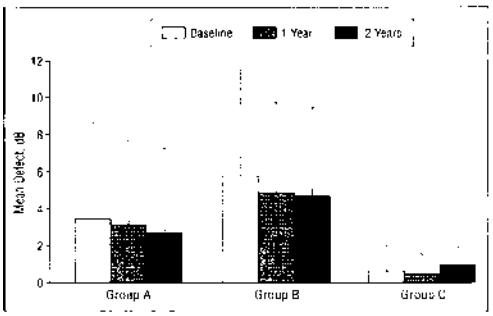

Figure 4. Mean defect values at baseline and 1 and 2 years after treatment in patients for whom *Helicobacter pylori* treatment was successful (group A); those for whom eradication of *H pylori* had failed (group B); and those who were *H pylori* negative at baseline (group C). Error bars indicate SD.

The only way to prevent visual loss with glaucoma is early diagnosis and treatment. At the present time, glaucoma therapy entails decreasing aqueous humor production, increasing fluid drainage, or a combination of these 2 using medical, laser, or surgical means.[4] This approach, however, treats only a risk factor (elevated intraocular pressure) and not the disease per se and thus fails to address the events that lead to the elevated intraocular pressure. A far more rational management of glaucoma in the future may be to direct our therapeutic interventions to the risk factors leading to glaucomatous neuropathy.[4] However, appropriate management of glaucoma requires a better understanding of the pathogenetic mechanisms involved.

As knowledge of glaucoma has accumulated, ophthalmologists have increasingly focused on prevention of the initial events that lead to optic nerve damage. Important to determining who will contract glaucoma are a number of risk factors, of which raised intraocular pressure is the best known. Several of these risk factors, such as advancing age and family history, are not modifiable. However, other risk factors may be modifiable, and their elimination may help to decrease incidence of blindness from glaucoma. Significantly, the populations of patients with glaucoma have a higher prevalence of systemic conditions such as arterial hypertension, obesity, and diabetes. To date, the increased prevalence of glaucoma in patients with these conditions has not been elucidated. Consequently, when the ophthalmologist treats glaucoma, he or she is treating a patient more likely to have, or be at greater risk for developing, vascular disorders. In addition, the most widely prescribed medicine to treat glaucoma (timolol, a topical β-blocker) may adversely affect serum lipid levels.[16] Thus, understanding how systemic disorders adversely affect glaucoma risk, as well as the effect of treatment of systemic conditions on glaucoma course and prognosis, may lead to better overall care of the patient with glaucoma.

In the first part of this study, by documenting a higher prevalence of *H pylori* in an OAG cohort than in an age- and sex-matched control group, we established for the first time a significant relationship between *H pylori* infection and glaucoma in a Greek ethnic cohort.[12] The *H pylori* infection was determined by histologic detection of organisms in mucosal biopsy specimens, which is considered the gold standard for the diagnosis of this infection. The *H pylori* prevalence of our control group is similar to that reported by other investigators who used serodiagnostic assay to evaluate Greek cohorts and other ethnic populations (frequency distribution, 34.1%-61.6%).[5]

It is worthwhile to consider whether the rate of *H pylori* infection in the control group has been negatively influenced by the coexistence of anemia. There is no evidence to suggest that anemia protects against development of *H pylori* infection. Anemic controls have been used before,[17] and the frequency of *H pylori* infection in the anemic control group matches that of the general population in Greece and that reported in other ethnic groups.[5] Furthermore, it is unlikely that individuals with iron-deficiency anemia are protected against *H pylori* infection because it is thought that the infection is actually associated with iron- and/or vitamin B_{12}–deficiency anemia.[18,19] In addition, eradication of *H pylori* infection may be associated with reversal of iron and/or vitamin B_{12} deficiency and improvement of anemia.[20]

In part 2 of the present study, we obtained an acceptable eradication rate of 83% by using a triple eradication regimen of omeprazole, clarithromycin, and amoxicillin for 1 week, which is standard practice in Europe. Similar eradication rates have been achieved by others.[7] Moreover, recurrence of duodenal and gastric ulcers was prevented after a successful *H pylori* eradication regimen for up to 1 year, findings that have also been reported for long-term follow-up periods.[21]

The present study established that patients in whom *H pylori* was successfully eradicated (group A) showed a statistically significant reduction in intraocular pressure at both clinical end points (1 and 2 years after treatment). This was not the case in groups B and C, despite the fact that all groups were maintained on the same antiglaucoma therapy they received at baseline. Thus, it seems that *H pylori* eradication was beneficial for the intraocular pressure control in these patients. Although a reduction of 1.2 mm Hg may not seem clinically significant (as opposed to statistically significant), we should

bear in mind the progressive nature of glaucoma and the fact that all patients were maintained on the same regimen for 2 years. Longer-term follow-up is required to validate the beneficial effect of *H pylori* eradication therapy.

Results of the present study suggest that eradication therapy may somehow improve the outflow facility of the eye. It should be noted, however, that the numbers of patients in groups B and C were small, and it may not, therefore, be possible to draw definitive conclusions. Future studies are needed to focus on the influence of *H pylori* in the outflow system of the eye. It is conceivable that *H pylori* induces the synthesis of various mediators (eg, cytokines), which may be detrimental to the outflow system of the glaucomatous eye. A fruitful line of future investigation would be comparison of the aqueous humor of patients with glaucoma who test positive for *H pylori* with those who test negative for the infection.

A similar improvement occurred in visual field data (mean SF and CLV at 1 and 2 years' follow-up). These results in group A are of interest because it is unusual for these parameters to improve over time. It should be noted that these changes occurred while patients were maintained on the same glaucoma regimen. Group B patients, on the other hand, showed a slight deterioration in SF and CLV mean values after 2 years of follow-up. These findings clearly suggest that patients in group A did better than those in group B over a period of 2 years. It is reasonable to hypothesize that it was the *H pylori* eradication that improved the parameters of these patients. These results are encouraging and suggest that possibly *H pylori* eradication should be attempted in patients with glaucoma. However, before this strategy becomes established practice, further evidence from large-scale prospective trials in various ethnic groups is needed to confirm our findings.

With regard to group C patients (*H pylori* negative at baseline), it is interesting to note that all baseline visual field indices were approximately within upper normal limit values and, in particular, 2 baseline visual field indices (MD and CLV) were significantly lower than in groups A and B. In addition, all glaucoma parameters showed a slight worsening over time, although these changes were not statistically significant. We do not know whether these observations will persist or progress over a longer period of follow-up. Because of the small number of glaucoma cases included in this group, future studies are needed to elucidate the natural course of *H pylori*–negative patients.

It should be noted that local administration of antiglaucoma drops results in systemic absorption through the nasal mucosa. It is known, for example, that within the upper GI tract, prostaglandins seem to heal ulcers by secretory inhibition rather than "cytoprotective" actions.[23] Therefore it is conceivable that latanoprost treatment (a prostaglandin analogue) may have influenced slightly the alimentary tract by inducing minor ulcer healing activity via inhibition of acid secretion. Timolol, a nonselective β-adrenoreceptor antagonist, is known to have failed to protect against ethanol-induced gastric lesions[24] and is not believed to meaningfully affect the GI tract. Pilocarpine has been known to affect GI motility, and potential effects on the GI tract include prolonged salivary secretion, diarrhea, and acid secretion.[24,25] Swallowed saliva secretion induced by pilocarpine might neutralize, at least in part, acid output. However, only 1 patient received pilocarpine in our group.[12] We think that it is the administration of omeprazole (strong proton pump inhibitor under all known stimuli)[22] that was the major contributor to ulcer healing in *H pylori*–positive and *H pylori*–negative patients.

The question arises exactly how *H pylori* infection influences the pathophysiology of glaucoma. The following possible mechanisms are suggested. *Helicobacter pylori* may promote the formation of L- and P selectin–dependent platelet-leukocyte aggregates in murine gastric microvessels, and *H pylori* may also induce platelet activation and aggregation[26,27] and atherosclerosis.[9] This phenomenon may play a part in the proposed relationship between *H pylori* and glaucoma, in which platelet activation and aggregation play a relevant pathophysiological role.[28,29] Alternatively, release of large amounts of variable proinflammatory and vasoactive substances such as cytokines (interleukin [IL] 1, IL-6, IL-8, IL-10, IL-12, tumor necrosis factor α, and interferon γ), eicosanoids (leukotrienes and prostaglandins), and acute-phase proteins (fibrinogen and C-reactive protein) following gastric colonization by *H pylori*[9,30,31] may be involved in a number of vascular disorders thought to be associated with the bacterium. These disorders include Raynaud phenomenon, idiopathic migraine, coronary heart disease,[9,30,31] and now possibly glaucoma (a similar cytokine profile seems to be involved in the pathogenesis of glaucoma disease).[32,33] Increased endothelin-1 (a potent constrictor of arterioles and venules), nitric oxide, and inducible nitric oxide synthase levels in peptic ulcer disease associated with *H pylori* infection[34] suggest that the resulting microcirculatory disturbance may be a major factor in the pathogenesis of local gastric mucosal ulceration and systemic damage, including glaucoma. Indeed, endothelin-1–induced vasoconstriction of the anterior optic nerve vessel and modulation of vascular tone in the ophthalmic artery by nitric oxide may be involved in the pathogenesis of glaucomatous damage.[4,35] *Helicobacter pylori* can coagulate blood by stimulating mononuclear cells. Under bacterial stimulation, mononuclear leukocytes produce a tissue factor–like procoagulant activity that, through the extrinsic pathway of blood coagulation, converts fibrinogen into fibrin. Thus, apart from its effect on fibrinogen level, *H pylori* has another activity (blood clotting), which might contribute to the pathogenesis of cardiovascular disorders[36] or glaucoma. Other factors such as the development of cross mimicry between endothelial and *H pylori* antigens[10] and excessive production of reactive oxygen metabolites and circulating lipid peroxides, which are associated with cardiovascular risk in patients with *H pylori* infection,[7,30] may also be involved indirectly in glaucoma pathophysiology. However, further studies are needed to elucidate these points.

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The Incidence of Helicobacter pylori Infection Is Not Increased Among Obese Young Individuals in Greece

[Alimentary Tract: Clinical Research]

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Abstracttoctoc

Goals: To identify possible risk factors affecting the acquisition of Helicobacter pylori (Hp) infection and to investigate whether the incidence of infection is higher among obese and overweight verus normal-weight young adults in Greece.

Study: Serum was obtained from 224 young male Navy recruits (mean age, 22.84 years) during their induction into the Hellenic Navy. An enzyme-linked immunosorbent assay to detect Hp-specific IgG serum antibodies, as well as gastroscopy with biopsy, were used to identify the infected individuals. A structured questionnaire was filled out for each subject regarding environmental conditions, socioeconomic conditions, dietary habits, and data related to their personal and family health history.

Results: H. pylori positivity rate was 27.23%. Univariate analysis recognized that the number of siblings in the same bedroom was significantly higher among Hp-positive than Hp-negative individuals. Logistic regression analysis showed that sharing the same bedroom with more than one sibling during childhood and consumption of fast food are independent predictors of Hp acquisition. The presence of obesity (body mass index >= 25 kg/m2) remained unrelated to the Hp status of the individuals.

Conclusions: These data suggest that the risk of Hp infection does not increase in overweight young persons. Sharing the same bedroom with more than one sibling during childhood is an important determinant in acquiring Hp infection. Increased fast food consumption could be an important source of the infection outside of the home.

Many risk factors have been connected with acquisition and progress of Helicobacter pylori (Hp) infection in humans. Although 50% of the general population is estimated to be affected, this percentage varies considerably with age, race, and nationality. 1 Overcrowded living conditions are well documented as an important determinant for infection with Hp by altering exposure to the bacterium. High level of educational, white-collar profession, and high monthly income, as indices of a higher socioeconomic level, have been negatively related to Hp infection. 2

Despite the recognized environmental, socioeconomic, and dietary factors, the variety of grades of response against Hp inoculation enforces the assumption that other pathogenetic factors also must participate. The immune system is designed to protect the individual from foreign substances and organisms. Subsequently, the host's immune response must be an important determinant of clinical outcome of Hp infection. 3 Overnutrition and obesity, besides their well-known complications including heart disease and diabetes mellitus, also can suppress a person's immune response. As a result, some studies recognize higher Hp infection rates in persons with high body mass index (BMI), especially in those who are young. 4,5

This study identifies possible risk factors affecting the acquisition of Hp infection among young Greek adults and investigates whether obesity and being overweight are factors that facilitate this acquisition.

MATERIALS AND METHODS

Subjects

The study population consists of 224 healthy male Hellenic Navy recruits (median age, 22.84 years; range, 20–30 years). All subjects were whites who came from different regions of the country. They were randomly selected from 600 men inducted into the Hellenic Navy at the Naval Base of Salamis. A blood sample was collected in February 2000 during their induction. After centrifugation, the serum sample was split into three aliquots, which were frozen at -70°C and thawed once before analysis.

Questionnaire: Study Variables

Data were collected from each recruit's childhood (1971–1981) and adolescence (1981–1991). During blood sampling, a questionnaire was filled out for each subject assessing biologic factors (the subject's personal health history) and the presence or absence of selected environmental factors (physical and socioeconomic) for both time periods. By personally interviewing the parents of the recruits, additional information was collected regarding the recruit's childhood and the parents' health history and family's socioeconomic status.

Physical information evaluated where they lived, the living conditions, and the food habits during both childhood and adolescence. Living in urban or rural area, in a detached house or an apartment, and the presence or absence, as well as the number, of siblings in the same bedroom categorized the recruits into different groups. According to information obtained from census reports of the Greek National Statistic Service, 6 the place where they lived was categorized as having many or few habitants and many or few buildings per square kilometer. The quality of food consumed daily grouped the subjects into vegetarians if they consumed mostly vegetables, fruits and milk products; they were not grouped as vegetarians if the ate mostly meat, carbohydrates, and sweets. The subjects' preference to homemade food or fast food from street vendors separated them into their respective dichotomous groups.

Results from our preliminary evaluation show that the current socioeconomic status of the recruits could not be assessed on the basis of personal educational and occupational levels. Mostly, recruits had just graduated from high school or university without any occupational experience. Conversely, their educational status mostly reflected the educational status of their parents and did not affect their current socioeconomic status. Because of these results, these two variables were not included in the subsequent analysis.

Characteristics of the socioeconomic status were correlated with the Hp status at presentation. The attained level of education of the recruits' parents, as well as father's profession, were evaluated as two distinct risk factors: parents who received formal education up to high school level were included in the first educational group, whereas college or university entrants formed the second group. Further categorization of each subject was performed according to the father's profession (blue- or white-collar workers). 7 Subjects were further grouped according to the family income during childhood as well as the current family income. Low income was considered as having earned up to 160,000 Greek drachmas/mo during childhood and up to 600,000 drachmas/mo being earned currently (approximately $400 and $1,500, respectively, at the time of study), whereas high income was considered as having earnings of over 160,000 and 600,000 Greek drachmas/mo, respectively.

The personal health condition of each subject was assessed. Specifically, by calculating the BMI, we created two distinct groups: the group of overweight individuals with BMI of 25 kg/m2 or more, and the group composed of lean individuals with BMI less than 25 kg/m2. The cutoff point of less than 25 kg/m2 or 25 kg/m2 and higher was proposed in the first Federal Obesity Clinical Guidelines released by the National Heart, Lung, and Blood Institute and the National Institute of Diabetes, Digestive and Kidney Diseases in 1998 as the most acceptable classification for obesity. 8 Personal history of gastrointestinal symptoms or diseases also was considered. Information regarding the presence of obesity during childhood was obtained after interviewing the parents and by reviewing the personal pediatric medical file of each individual. The pediatrician's indication of the presence of obesity categorized the subjects into the proportional groups.

Having at least one parent with a positive history of gastrointestinal symptoms and the presence or absence of obesity were the data used to evaluate family health status.

Positive history of tumors, tuberculosis, easily acquired infections, recurrent fungal or herpes infections, known allergies, or problematic reactions to vaccinations were considered as indicators of a compromised immunologic status for both the studied individuals and their parents. None of the recruits studied had a history of infection with human immunodeficiency virus, and lymphocyte number was within the normal range for all. Age and serum levels of glucose, urea, creatinine, and uric acid were separately evaluated.

Determination of H. pylori Statustoctoc

Titers of Hp-specific IgG antibodies, as well as serum levels of glucose, urea, creatinine, and uric acid, were determined in the Department of Immunology of Naval Hospital of Athens. For serologic determination of the specific antibody against Hp, an enzyme-linked immunosorbent assay was used (Hp IgG Elisa Kit, Hycor Biomedical GmbH, Kassel, Germany; interassay coefficient of variation, 7%; intra-assay coefficient of variation, 4.3%; sensitivity, 0.1 IU/mL). Levels of IgG were categorized as seropositive for Hp if the optical density titer of IgG antibodies to Hp was above 0.5. Normal ranges were as follows: for blood glucose, 60 to 110 mg/dL; serum urea, 10 to 40 mg/dL; creatinine, less than 1.2 mg/dL; and uric acid, up to 5.3 mg/dL. All seropositive subjects underwent endoscopy in the Department of Gastroenterology, Naval Hospital of Athens. Along with the examination, two biopsy samples, one from the antrum and one from the corpus, were obtained from each individual for verification of Hp infection. Subjects with both serosurveillance and biopsy-positive results were finally considered as Hp-contaminated individuals.

Statistical Analysestoctoc

To examine whether the Hp-affected members differed from the nonaffected members for each study variable, the [chi]2, Fischer exact, and Student t tests were used. With the exception of the continuous variables, the correlation of the rest of the covariates with respect to Hp positivity in the examined groups was evaluated by logistic regression analysis from the SPSS statistical package version 8 (SPSS, Chicago, IL, U.S.A.).

RESULTStoctoc

Of 224 subjects, serologic testing identified 71 Hp-specific IgG-positive individuals. Endoscopy and biopsy verified the presence of infection in 61 subjects, resulting in a prevalence of 27.23%.

Tables 1 and 2 show the differences between Hp-affected and Hp-nonaffected individuals for the evaluated parameters. H. pylori-positive members had lived in places with fewer inhabitants per square kilometer than did Hp-negative members during both childhood and adolescence ($p = 0.04$ and 0.03, respectively). The density of buildings (buildings per square kilometer) also was significantly less among the Hp-negative members than among Hp-positive members but only during the second time period ($p = 0.01$). From our preliminary analysis (data not shown), we identified that living as a child, but also as a young adult, in these crowded areas was related to living in a city rather than in a village and in an apartment instead of a detached house ($p < 0.001$). This "crowded" urban living environment reflected the father's white-collar profession, higher family educational level, and higher family income ($p = 0.01$, 0.03, and 0.001, respectively).

TABLE 1. Correlation of the variables reflecting the living conditions during childhood and adolescence to H. pylori status* $p = 0.04$. †$p = 0.01$. ‡$p = 0.03$. Hp indicates H. pylori.

TABLE 2. Correlation of variables reflecting personal and family data with H. pylori status* $p = 0.06$. BMI indicates body mass index; Hp, H. pylori.

Univariate analysis recognized significantly higher rates of Hp infection among the individuals who were sharing their bedroom with two or more siblings (a total of three or more persons in the same bedroom) than among individuals with one or no siblings in the same bedroom. This finding was constant during both childhood and adolescence (p = 0.01 and 0.03, respectively). According to our preliminary evaluation (data not shown), the number of siblings in the same bedroom during adolescence and early adulthood, although it also was related to increased Hp infection rates (p = 0.03), was connected mainly to lower professional and educational levels of the family (p = 0.03 and 0.004, respectively) and lower current family income (p = 0.01). As a result, its relation to Hp positivity lost its significance when it was controlled for these parameters. To the contrary, the presence of more than two siblings in the same bedroom during childhood was not related to any of the rest of the variables except increased Hp positivity rates (p = 0.01). Among the rest of the variables, only fast food consumption had a tendency to be higher among Hp-positive individuals (p -- 0.06), whereas BMI remained unrelated to Hp status (p = 0.53).

Logistic regression analysis recognized both excess fast food consumption (p = 0.02) and the presence of two or more siblings (in addition to the studied individual) in the same bedroom during childhood (p = 0.03) as independent predictors of Hp acquisition. There also was a tendency for Hp positivity to be correlated with low educational level of parents (p = 0.06;Table 3). Notice that obesity during childhood and obesity among parents revealed a positive correlation with Hp positivity, but, to the contrary, BMI of 25 kg/m2 or more had a negative relation to Hp positivity.

TABLE 3. Logistic regression with H. pylori positivity as dependent variableBMI indicates body mass index; CI, confidence interval; SE[beta], standard error of the coefficient value; [beta], coefficient value.

DISCUSSIONtoctoc

Many different factors have been proposed as risk factors for Hp acquisition.

Significant variation in the extent of Hp infection has been identified by race, sex, and nationality as well as by developed versus developing countries. 1,9,10 In our study, all subjects were white Greek men, and the recognized prevalence of Hp positivity (27.23%) was similar to the age-specific seroprevalence observed in other developed countries (~20%).

Environmental and socioeconomic conditions may play an important role in the acquisition of the infection. Ample epidemiologic information shows that infection with Hp occurs more frequently in socioeconomically deprived populations living in crowded places with degraded hygienic conditions and, conversely, with lower frequency in persons of high socioeconomic status. 11 14 In our study, living in an area with high population and buildings densities was related to decreased Hp acquisition. When the population density (population per building per square kilometer) was calculated, the value was higher for Hp-negative than Hp-positive persons during their childhood. This value became higher for the Hp-positive than Hp-negative persons during their adolescence (Table 1). The reason for this inversion was that in the Hp-negative group of recruits, the increment in the number of buildings per square kilometer from childhood to adolescence was not accompanied by the proportional increment in the number of inhabitants per square kilometer. To the contrary, among the Hp-positive subjects, the increments of the population and building densities were proportional, resulting in a constant value of population density (population per building per square kilometer) between the two time periods. This finding indicates that the areas where the former group was living were areas under development where more social buildings, stores, and offices were constructed and a better quality of life was established during these years. The prevalence of Hp acquisition was decreased in this living environment in comparison with less crowded places (p = 0.03), indicating that living in a crowded urban area during these years (1971–1991) in Greece was directly connected with a better socioeconomic level. 6

Our results confirm that an increased number of siblings during childhood, but also during adolescence and early adulthood, is an important predictor for Hp acquisition. In agreement with Jimenez-Guerra et al., 15 we identify that when the number of siblings in the same bedroom was equal to or higher to three (two siblings and the studied individual), the hazard of Hp acquisition was significantly increased. Logistic regression analysis recognized only the number the siblings during childhood, and not that during adulthood, as an independent predictor. So, it is important to understand possible differences in the interpretation of these two parameters. In this study, the increased number of siblings (three or more) in the same bedroom during adolescence and early adulthood mainly reflected a low socioeconomic status. As a result, the identified correlation with increased Hp infection rates lost its

significance when it was adjusted for socioeconomic parameters. To the contrary, the presence of more than two siblings in the same bedroom during childhood was not related to any of the rest of the variables except increased Hp positivity rate. So, it did not reflect the socioeconomic status of the individuals but may underline the intrafamilial person-to-person mode of transmission of infection.

Our current findings emphasize the difference between crowding in the area of living (a possible indicator of economically advanced area) and crowding inside the household, an indicator of poor economic resources.

Dietary factors in Hp-associated gastroduodenal disease remain uncertain. Adequate intake of antioxidant micronutrients (particularly vitamin C and [beta]-carotene) and higher consumption of fruits and vegetables were negatively associated with infection. Little evidence was found for an important role of fat or protein intake, although patients with eating disorders (anorexia nervosa, bulimia nervosa) do not have an increased rate of Hp infection. 11,16,17 In Japan, the presence of Hp infection has a significant correlation with increased intake of high-sodium food, although an increased prevalence of infection has been associated with increased consumption of food from street vendors. 18,19 In our series, the consumption of fast food instead of homemade food was proved to be an important factor for the acquisition of infection. Neither the kind of food (vegetarian or otherwise) nor the frequency of the meals yielded any significance. The quality of the food may affect resistance to Hp infection, and nutritional factors may play a role in determining susceptibility to the bacterium. 20 Excessive fast food consumption could be a possible source of infection outside of the home, which could be partially responsible for the 24 Hp-positive subjects (29.2%) identified in our study of 82 subjects who had no siblings.

Although environmental, socioeconomic, and dietary factors clearly are important in acquisition of the infection, data from many studies suggest that host defense, as well as factors affecting host resistance, also determine whether infection will be acquired. Obesity has been linked to alteration of immune function and to a higher incidence of infection and several types of cancer. 21–23 Although our assessment does not reveal any correlation between BMI and Hp positivity, logistic regression identifies a positive correlation between obesity during childhood or having obese parents and Hp positivity among the recruits. The finding that the correlation became negative between current BMI and Hp positivity may indicate that obesity is a factor that could promote Hp acquisition during childhood under specific conditions. This finding is in accordance with the results of the EUROGAST study group 4 reporting that, possibly, BMI at a young age more accurately reflects the relevant social class than does BMI at an older age. However, in our series with a specific age range (20 to 30 years), the hypothesis that obese or overweight persons could present a higher rate of Hp acquisition was not confirmed. Similarly, Strachan 24 identifies that seropositivity for Hp is significantly associated with poorer socioeconomic status but not with age, smoking, or BMI. Pilott et al. 25 conclude that presence of anti-Hp antibodies is not affected by the nutritional status and BMI of the individuals. Interestingly, Scapa et al. 26 recognize that in patients with morbid obesity, the incidence of Hp was low for unknown reasons.

In conclusion, we could not identify higher prevalence of Hp infection among the overweight or obese persons of our population. Sharing the same bedroom with two or more siblings during childhood was an independent predictor for Hp acquisition. This was not true for adolescence or early adulthood. As a result, these time periods should be separately interpreted in studies evaluating the possible risk factors for Hp acquisition.

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Key Words: Helicobacter pylori; Obesity; Childhood; Siblings

TABLE 1

Variable	Hp-negative (n = 163)	Hp-positive (n = 61)
Living place (childhood)		
Area		
Urban	140 (81.5%)	52 (85.2%)
Rural	23 (19.5%)	9 (14.8%)
Residence		
Detached house	66 (40.5%)	23 (37.7%)
Apartment	97 (59.5%)	38 (62.3%)
Inhabitants/km^2 (±00)	4,188.1 ± 3190	3,193.1 ± 3,316.4*
Buildings/km^2	582.6 ± 439.7	467.3 ± 457.4
Population/ building/km^2 (±00)	11 ± 10.4	10.9 ± 8.7
Living conditions (childhood)		
Siblings in the same bedroom		
No	59 (36.2%)	24 (39.3%)
Yes	104 (63.8%)	37 (60.7%)
Number of siblings in the same bedroom with the studied individual		
0 or 1	154 (94.5%)	51 (83.6%)†
≥2	9 (5.5%)	10 (16.4%)
Income (drachmas/mo)		
<160000	44 (27%)	22 (36.1%)
≥160000	119 (73%)	39 (63.9%)
Living place (adolescence and early adulthood)		
Area		
Urban	147 (89.5%)	51 (83.6%)
Rural	17 (10.5%)	10 (16.4%)
Residence		
Detached house	59 (36.2%)	22 (36.1%)
Apartment	104 (63.8%)	39 (63.9%)
Inhabitants/km^2 (±00)	4484.1 ± 3148	3453.8 ± 3352‡
Buildings/km^2	662 ± 447	499 ± 485†
Population/building/ km^2 (±00)	9.4 ± 8.2	10.2 ± 6.5
Living conditions (adolescence and early adulthood)		
Siblings in the same bedroom		
No	67 (41.1%)	29 (47.5%)
Yes	96 (58.9%)	32 (52.5%)
Number of siblings in the same bedroom with the studied individual		
0 or 1	156 (95.7%)	53 (86.9%)‡
≥2	7 (4.3%)	8 (13.1%)
Income (drachmas/mo)		
<600000	93 (57.1%)	39 (63.9%)
≥600000	70 (42.9%)	22 (36.1%)

*p = 0.04.
†p = 0.01.
‡p = 0.03.
Hp indicates *H. pylori*.

TABLE 2

Variable	Hp-negative (n = 163)	Hp-positive (n = 61)
Personal data		
Age	22.6 ± 2.6	22.4 ± 2.4
Obesity		
Yes	28 (17.2%)	14 (23%)
No	135 (82.8%)	47 (77%)
Vegetarian		
Yes	50 (30.7%)	18 (29.5%)
No	113 (69.3%)	43 (70.5%)
Fast food consumer		
Yes	24 (14.7%)	15 (24.6%)*
No	139 (85.3%)	46 (75.4%)
History of gastrointestinal complaints		
Positive	28 (17.2%)	12 (19.7%)
Negative	135 (82.8%)	49 (80.3%)
History of immunology disturbances		
Positive	54 (33.1%)	18 (29.5%)
Negative	109 (66.9%)	43 (70.5%)
BMI		
<25 kg/m^2	61 (37.4%)	26 (42.6%)
≥25 kg/m^2	102 (62.6%)	35 (57.4%)
Glucose (mg/dL)	104.9 ± 12.6	103.5 ± 8.3
Urea (mg/dL)	34.7 ± 7.4	34.1 ± 7.2
Creatinine (mg/dL)	0.8 ± 0.1	0.8 ± 0.1
Uric acid (mg/dL)	5.7 ± 1.1	5.9 ± 0.9
Family data		
Father's profession		
"Blue collar"	76 (46.6%)	30 (49.2%)
"White collar"	87 (53.4%)	31 (50.8%)
Working mother		
Yes	61 (37.4%)	25 (41%)
No	102 (62.6%)	36 (59%)
Family education		
High	37 (22.7%)	8 (13.1%)
Low	126 (77.3%)	53 (86.9%)
Parents obese		
Yes	60 (36.8%)	25 (41%)
No	103 (63.2%)	36 (59%)
History of gastrointestinal symptoms		
Positive	44 (32.4%)	13 (21.3%)
Negative	119 (73%)	48 (78.7%)
History of immunology disturbances		
Positive	31 (19.1%)	12 (19.6%)
Negative	132 (81%)	49 (80.4%)

*p = 0.06.
BMI indicates body mass index; Hp, *H. pylori*.

TABLE 3

Covariates	β	SE_β	*p* value	95% CI
Living in urban area (childhood)	−0.15	0.3	0.59	0.47–1.53
High income during childhood	−0.57	0.38	0.15	0.2–1.23
Living in apartment (childhood)	−0.55	0.46	0.23	0.22–1.43
Presence of siblings in the same bedroom (childhood)	+0.46	0.41	0.26	0.67–3.6
≥3 siblings in the same bedroom (childhood)	+1.44	0.68	0.03	0.06–0.89
Living in apartment (adulthood)	+0.41	0.45	0.36	0.7–2.91
High income during adulthood	−0.22	0.34	0.52	0.39–1.53
Presence of siblings in the same bedroom (adulthood)	+0.47	0.4	0.26	0.28–1.37
≥3 siblings in the same bedroom (adulthood)	+0.34	0.76	0.64	0.15–2.88
Presence of obesity during childhood	+0.5	0.45	0.24	0.77–3.7
BMI ≥25 kg/m^2	−0.48	0.37	0.19	0.7–3.18
Fast food consumer	+0.99	0.43	0.02	0.18–0.97
Vegetarian	−0.007	0.37	0.79	0.43–1.88
High family education	−0.66	0.36	0.06	0.26–1.007
"White collar" father's profession	−0.32	0.38	0.39	0.41–1.76
Parents obesity	+0.44	0.39	0.25	0.3–1.33
Positive personal history of gastrointestinal symptoms	+0.51	0.46	0.27	0.6–1.89
Positive family history of gastrointestinal symptoms	+0.5	0.38	0.25	0.44–2.01

BMI indicates body mass index; CI, confidence interval; SE_β, standard error of the coefficient value; β, coefficient value.

positive result is recorded within 1 min if two pink lines, i.e. test nd control, are visible and a negative result if the control line is bserved only in the viewing window. The Pyloriset EIA-G is an IA that measures IgG serum antibodies to *Helicobacter pylori*. A utoff value of ≥300 U/ml defined a positive test. Each test, i.e. LO-Test, gram-stained smears, histological examination, Test-ack Plus and Pyloriset EIA-G, was performed by an investigator ho was blinded to the results of the other tests. The gold standard r *Helicobacter pylori* positivity was defined as positive results in t least two of the three reference tests, i.e. histological examina-ion, CLO-Test and Gram smear, while the gold standard for *elicobacter pylori* negativity was defined as negative results in ll three tests. Patients with a positive result in only one reference est were excluded from further analysis.

Sensitivity, specificity, predictive values and diagnostic accu-acy were determined separately for each serological test, based n comparison with the gold standard. Qualitative data were as-essed by the chi-square test with Yates' correction. A P value of 0.05 was considered significant.

Results and Discussion

One hundred fifty-three of a total of 160 consecutive dyspeptic patients fulfilling the inclusion criteria were tudied. They included 94 men and 59 women with a nedian age of 47 years, (range, 17–85 years). Eighty pa-ients had duodenal ulcer, 12 gastric ulcer and 61 nonul-er dyspepsia. Seventy-four patients (40 men, 34 women) were younger than 45 years (median age, 34 years; ange, 17–45 years), including 34 patients with duodenal lcer and 40 with nonulcer dyspepsia. Using the gold tandard, 110 (72%) patients were defined as *Helicobac-er pylori* positive and 43 as *Helicobacter pylori* nega-ive.

The results of the TestPack Plus and the Pyloriset EIA-G are shown in Table 1. There were no statistically ignificant differences in the performance characteristics f TestPack Plus as compared with the Pyloriset EIA-G chi-square <0.4, DF=1, P>0.5). Comparison of the estPack Plus's performance in patients younger than 45 years versus its performance in patients older than 45 years showed a significantly different negative pre-dictive value (chi-square=6.2, two-tailed P=0.008). The ame comparison for the Pyloriset EIA-G showed signif-

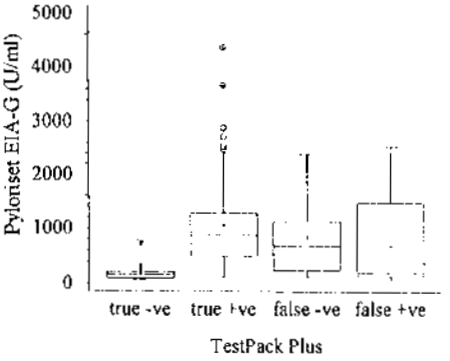

Fig. 1 Box-and-whisker plots of serum *Helicobacter pylori* IgG antibody titers (Pyloriset EIA-G) in patients with true positive, true negative, false negative and false positive results in the Test Pack Plus test. The median value is represented as a vertical line inside the box. Outliers, i.e. points more than 1.5 times the interquartile range from the end of the box, are shown as open circles. It is evident that most (10/14) of the patients with false-negative TestPack Plus results had serum IgG antibody titers higher than 300 EIA (Pyloriset EIA-G) units

icantly different specificity (chi-square=5.0, two-tailed P=0.02) and negative predictive values (chi-square=6.7, two-tailed P=0.01). The TestPack Plus had a high positive predictive value (93%), but a relatively low negative predictive value (72%). To investigate whether this low negative predictive value was related to a low *Helicobacter pylori* IgG antibody titer, we compared IgG titers obtained with the Pyloriset EIA-G with titers obtained in the true-positive, true-negative and false-negative TestPack Plus tests. Figure 1 shows that most (10/14) of the patients with false-negative TestPack Plus results had IgG antibody titers to *Helicobacter pylori* higher than the cutoff value for a positive Pyloriset EIA-G test (≥300 EIA U/ml). Therefore, only four patients were falsely identified as negative by both serological tests. Furthermore, five of seven patients with false-positive TestPack Plus tests had serum IgG antibody ti-

Table 1 Results of the TestPack Plus and the Pyloriset EIA-G for diagnosis of *Helicobacter pylori* infection in untreated patients. A combination of the biopsy-based tests (CLO-Test, histological examination, Gram smear) was used as the gold standard

Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	ODA (95% CI)
All patients					
TestPack Plus	87 (80–93)	84 (69–93)	93 (87–97)	72 (58–84)	86 (80–91)
Pyloriset IgG	91 (84–96)	88 (75–96)	95 (89–98)	79 (65–90)	90 (84–94)
Patients ≤45 years					
TestPack Plus	88 (75–96)	90 (74–98)	93 (80–89)	85 (68–95)	89 (80–95)
Pyloriset IgG	93 (81–99)	97 (83–100)	98 (87–100)	91 (76–98)	95 (87–99)
Patients >45 years					
TestPack Plus	87 (76–94)	67 (57–77)	94 (84–98)	47 (36–58)	84 (74–91)
Pyloriset IgG	90 (80–96)	67 (57–77)	94 (85–98)	53 (42–64)	86 (76–93)

95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value; ODA, overall diagnostic accuracy

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CONCISE ARTICLE

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Evaluation of a Single-Step Serological Assay for Laboratory Diagnosis of *Helicobacter pylori* Infection

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Abstract A rapid, single-step, in-laboratory qualitative test for the detection of IgG antibodies to *Helicobacter pylori* in serum (TestPack Plus; Abbott Laboratories, Germany) was evaluated. This test may be used as an alternative to enzyme immunoassays (EIAs). Of 153 adult patients, 110 were defined as *Helicobacter pylori* positive and 43 as *Helicobacter pylori* negative by the gold standard, a combination of three tests. The performance characteristics of the TestPack Plus, i.e. sensitivity, specificity, and positive and negative predictive values, were not significantly different from the corresponding values obtained with an EIA used for comparative purposes, the Pyloriset EIA-G test (Orion Diagnostica, Finland). The high positive predictive value (93%) of the TestPack Plus single-step serological test makes it a valuable tool for rapid in-laboratory screening purposes, especially in countries with a high prevalence of *Helicobacter pylori* infection.

Introduction

The detection of *Helicobacter pylori* can be accomplished by invasive techniques, which require endoscopy, and noninvasive methods [1]. According to the European *Helicobacter pylori* Study Group [2], *Helicobacter pylori*-infected dyspeptic patients younger than 45 years who are without alarming symptoms such as weight loss, dysphagia and anemia may be managed without upper gastrointestinal endoscopy. Although this strategy is cost-effective, its usefulness depends on the accuracy of the diagnostic method used to detect *Helicobacter pylori* infection. The noninvasive methods recommended by the European *Helicobacter pylori* Study Group [2] are the ^{13}C-urea breath test and detection of *Helicobacter pylori* antigen in stool. However, these tests are relatively expensive and are not yet universally available in Western and developing countries. Several commercially available enzyme immunoassay (EIA) kits that detect anti IgG antibodies quantitatively have been used over the last decade [3, 4, 5]. In the present study, we evaluated a rapid, single-step test for detection of *Helicobacter pylori* antibodies in serum (TestPack Plus; Abbott Laboratories, Germany) and compared it with a commercially available EIA kit (Pyloriset EIA-G; Orion Diagnostica, Finland) that detects *Helicobacter pylori* IgG antibody in serum. Both tests were performed in the laboratory.

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Materials and Methods

Patients previously treated for *Helicobacter pylori* infection were excluded, as were patients who had received antibiotics or acid suppression therapy in the last 2 months before the study. Other exclusion criteria were malignancy, pregnancy and prior gastric surgery. The protocol of the study was approved by the ethics committee of our institution in June 1999.

A total of five gastric mucosa biopsies were obtained during endoscopy. One biopsy from the antrum was used to perform a rapid urease test (CLO-Test; Delta West PTCL, Australia). The result of this test was read at 2 h by a single trained observer. Two biopsies from the antrum were crushed separately between two microscope slides, and smears were Gram stained and evaluated by a bacteriologist for the presence of spiral gram-negative bacteria [6]. The remaining two biopsies, one from the antrum and the other from the gastric body, were sent to the pathology department. Modified Giemsa staining was used to detect *Helicobacter pylori* in biopsy specimens.

Antibody testing for *Helicobacter pylori* was performed by means of a rapid single-step serological test (TestPack Plus) and an EIA kit (Pyloriset EIA-G) in sera obtained before endoscopy. The TestPack Plus is a membrane-based qualitative immunochemical assay that detects IgG serum antibodies to *Helicobacter pylori*. When serum from a capillary tube is applied on the test card,

ters lower than 300 Pyloriset EIA-G units (Fig. 1, Table 1), and therefore only two patients were falsely identified as being positive by both tests.

The screen-and-treat strategy can only be applied if noninvasive, inexpensive and rapid diagnostic tests are widely available to general practitioners. Although the Maastricht II–2000 Consensus Report has suggested urea breath and stool antigen tests are the best screening methods [2], serological investigation probably is the test of choice because, despite being less expensive, it is still the most widely available method to screen untreated young dyspeptic patients for *Helicobacter pylori* infection. This is the case not only for the developing countries but also for Western countries [7]. Serological tests occasionally perform poorer than expected in elderly patients [8], probably because of relative immunoparesis, but this does not adversely affect the screen-and-treat strategy. Rapid in-laboratory serological tests offer an advantage both to general practitioners desiring quick results and to small laboratories that do not have the volume of *Helicobacter pylori* testing to justify the use of an EIA kit.

We have evaluated the performance of a new, rapid serological test (TestPack Plus) and compared the results with a commercially available send-out EIA (Pyloriset EIA-G). We recognize that the validation of a test is highly dependent on the reliability of the reference method used and that no single test is accurate enough to characterize a patient's *Helicobacter pylori* status unequivocally [8]. Therefore, we employed the combination of histological examination, the CLO-Test and Gram smears to define *Helicobacter pylori* status using biopsies from the gastric body and antrum to reduce sampling errors [9]. In our study we used the Pyloriset EIA-G kit to measure IgG antibodies to *Helicobacter pylori*. The new version of the Pyloriset EIA-G test kit was introduced in 1995. Since then, it has been validated in several European studies [5, 7, 10, 11, 12, 13, 14]. In these studies the median sensitivity of the Pyloriset EIA-G was 94% (range, 91–100%), specificity 90% (78–98%), positive predictive value 95% (92–99%) and negative predictive value 92% (72–100%). Our data from Greek patients are within these ranges. The high incidence of *Helicobacter pylori* infection (72%) detected in our study can be explained by the fact that the prevalence of *Helicobacter pylori* infection at the age of 45 years is about 40% in many European countries [15], including Greece [16]. In addition, 60% of our patients had peptic ulcer disease and had been referred by their general practitioner for endoscopic evaluation. This high prevalence of *Helicobacter pylori* infection among patients referred to endoscopy is consistent with published reports [5, 7] and helped us obtain clinically meaningful positive predictive values for diagnosis of *Helicobacter pylori* infection, because positive predictive value drops dramatically if the prevalence of *Helicobacter pylori* infection is within the range of 10–30%. It is important, therefore, to emphasize that this test should be used in populations where the prevalence of *Helicobacter pylori* infection is high.

Several rapid tests using serum [17, 18] or whole blood [13, 19–21] have recently been developed to facilitate the screen-and-treat strategy for *Helicobacter pylori* infection. These tests are simple to perform in the laboratory or in the physician's office. The result is read within a few minutes. These tests are less expensive than EIA testing [22]. In agreement with published reports [17, 18], our data show that, among patients in whom the prevalence of *Helicobacter pylori* infection is high, the performance of rapid serological tests is comparable to that of serological EIAs, except that the negative predictive value is lower. This is because patients falsely identified as being *Helicobacter pylori* negative by a rapid serological test may have a high (positive) IgG antibody titer when tested by EIA (Fig. 1).

Even though the overall diagnostic accuracy of the TestPack Plus is not ideal, the positive predictive value of the test is high. If one of the major purposes of serological testing is to eliminate the need for endoscopy in young *Helicobacter pylori*-positive dyspeptic patients, then patients identified as *Helicobacter pylori* positive by the test could reliably be assumed to be truly positive. They could then be treated for the infection without having to undergo endoscopy. Only a very small proportion of negative patients will falsely test positive (4.1% for TestPack Plus) and will thus not undergo the endoscopy that would otherwise be indicated. The larger numbers of infected young patients who falsely test negative would simply undergo endoscopy as normal and would not be compromised (6.8% for TestPack Plus). Missing positive patients would lead to harm only if such patients then did not receive appropriate therapy.

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ORIGINAL ARTICLE

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Performance of Two Immunosorbent Assay Kits for the Detection of Serum Immunoglobulin G to *Helicobacter pylori* in Untreated Greek Patients

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Ladas SD, Malamou H, Triantafyllou K, Varzakakos I, Georgopoulos S, Giota G, Rokkas T, Spiliadi C, Raptis SA. Performance of two immunosorbent assay kits for the detection of serum immunoglobulin G to *Helicobacter pylori* in untreated Greek patients. Scand J Gastroenterol 2002;37:512–516.

Background: The performance of serological methods used to detect *Helicobacter pylori* varies with the ethnicity and prevalence of the infection in the community. We have prospectively evaluated the performance of two commercially available serum enzyme immunoassays (EIA) detecting *H. pylori* immunoglobulin G (IgG) antibodies in the sera of untreated Greek patients. **Methods:** One-hundred-and-thirty consecutive untreated dyspeptic patients underwent endoscopy with biopsies from the gastric body ($n = 2$) and antrum ($n = 2$). Serum samples were also obtained from each patient. Serum *H. pylori* IgG antibody titres were determined with two EIA kits (Pyloriset EIA-G and Milenia *H. pylori* IgG). Sensitivities, specificities and optimal cut-off values of serum EIAs were determined for the population under investigation by using receiver operating characteristic (ROC) curve analysis and histology as gold standard. **Results:** Ninety-seven patients were defined *H. pylori*-positive and 33 negative by histology. ROC curve analysis for the Pyloriset kit yielded 86% (95% CI, 78%–92%) sensitivity and 85% (68%–95%) specificity at an optimal cut-off value of ≥358 units/ml. The respective values for the Milenia kit were 86% (78%–92%) and 82% (65%–93%) at an optimal cut-off value of ≥51 units/ml. The suggested cut-off values of the manufacturers for Pyloriset and Milenia kits are ≥300 and ≥44 EIA units, respectively, which yield 2% and 4% higher sensitivity, but 9% lower specificity for both EIA kits. **Conclusions:** Both serum EIA kits performed well in our study. Our data show that EIA cut-off values should be optimized for the population under investigation.

Key words: *Helicobacter pylori*; Milenia; Pyloriset; receiver operating characteristic curve analysis; serum enzyme immunoassay (EIA); serum IgG antibodies

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Helicobacter pylori is causally associated with chronic gastritis and peptic ulcer disease (1, 2) and is epidemiologically related with gastric cancer (3). Detecting *H. pylori* infection is therefore clinically (4) and epidemiologically (5) important.

Enzyme-linked immunosorbent assays (EIAs) that measure serum immunoglobulin G (IgG) to *H. pylori* are the cheapest and most widely available diagnostic methods used to screen untreated patients for *H. pylori* infection in general practice (6–8) and to investigate the *H. pylori* infection rate in population-based epidemiological studies (9). Most of the commercially available kits are highly sensitive and specific in determining *H. pylori* status (10, 11). However, the performance of EIA kits varies with the antigens chosen, the population from which the reference sera are drawn, the ethnicity of the population under investigation (12) and the age of the patients (13, 14). The cut-off value for a positive test must therefore be adjusted before an EIA kit is used in a certain country (15).

In the present study, we aimed to evaluate the performances of two commercially available EIA kits in detecting serum IgG antibodies to *H. pylori* in untreated patients. Both EIA kits are manufactured in Europe. Pyloriset EIA-G has been imported to Greece for more than 5 years, but its performance has never been evaluated with sera of Greek adults. Furthermore, there is no national or international formal evaluation of Milenia *H. pylori* IgG EIA, which has been on the market in Greece for more than 4 years.

Methods

Patients

One-hundred-and-thirty consecutive, untreated dyspeptic patients referred for endoscopy were studied. Patients who had taken proton-pump inhibitors, bismuth compounds or antibiotics within the previous 2 months were excluded, as well as those who had previously been treated for *H. pylori* infection. Other exclusion criteria were malignancy, pregnancy and prior gastric surgery. Informed consent was obtained from each patient. The protocol of the study was approved by the Ethics Committee on Human Studies, Dept. of Internal Medicine, University of Athens, in November 1999.

Diagnostic assays

All patients underwent upper gastrointestinal endoscopy and a total of four biopsies, two from the gastric body and two from the antrum, immersed in formalin and sent to the pathology department. A modified Giemsa staining was used to detect *H. pylori* in biopsy specimens by two experienced pathologists blinded to the results of endoscopy and serology. The Warthin-Starry technique was employed for negative cases. Patients were classified as *H. pylori*-positive if at least one biopsy was positive and *H. pylori*-negative when all biopsies were negative.

Before endoscopy, venous blood (10 ml) was obtained. Serum samples were prepared by allowing the blood to clot at room temperature and aspirating the supernatant. The sera were then sent to the microbiology department and stored at −20 °C until the antibody determination for *H. pylori* was performed batchwise.

Serum antibody (IgG) testing for *H. pylori* was performed by means of two EIA kits (Pyloriset EIA-G, Orion Diagnostica, Espoo, Finland and Milenia *H. pylori* IgG, DPC Bierman GmbH, Germany). They were used according to the manufacturers' instructions. EIA units were calculated from standard curves. *H. pylori* antibodies (IgG) were measured in duplicate in each serum. The test was repeated the next day in cases with an interassay variation of more than 10%. According to the Pyloriset test kit, a cut-off value of ≥300 units/ml defined a positive test. The corresponding cut-off value for the Milenia kit was ≥44 units/ml. All analyses were done by two bacteriologists. One of them analysed sera with the Milenia kit and the other with the Pyloriset kit. Each of the two bacteriologists was blinded to the results of the other one, and they were both unaware of the results of endoscopy and histology.

Statistics

Sensitivities, specificities, predictive values and diagnostic accuracy were determined separately for each serum EIA kit, by taking the results of histology as gold standard. Diagnostic accuracy was calculated as the percentage of the patients who were correctly scored (true positive plus true negative) among all patients tested.

Results were stored in a dBASE software (Microsoft Access 2000, Microsoft Corp.) and analysed by statistical package Statgraphics Plus 3.0 (Manugistics Inc., Statistical Graphics Corp., Rockville, USA). A receiver operating characteristic (ROC) curve analysis was used (MedCalc 6.00, MedCalc Software, Mariakerke, Belgium) to determine the best cut-off value for each EIA kit, which corresponded with the highest accuracy, i.e. minimal false-negative and false-positive rate. Results were expressed with 95% confidence intervals (CI). A P value of <0.05 indicated statistical significance.

Results

Sera from 130 consecutive dyspeptic patients (82 men, 48 women, age range 17–85 years, median 50 years) fulfilling the inclusion criteria were analysed. Fifty-six patients had non-ulcer dyspepsia, 52 duodenal ulcer, 7 gastric ulcer and 15 oesophagitis. Overall, 97 (75%) patients were defined *H. pylori*-positive and 33 negative by histology (gold standard).

Performance of the Pyloriset EIA-G

At the manufacturer's suggested cut-off value of ≥300 EIA units for the Pyloriset EIA kit, there were 11 false-negative and 8 false-positive patients, resulting in 88% (80%–94%) sensitivity, 76% (58%–89%) specificity and 85% (79%–91%) overall diagnostic accuracy (Table I). ROC curve analysis (Fig. 1) showed that the optimal cut-off value was ≥358 EIA units. At this cut-off value there were 13 false-negative and 6 false-positive patients (Fig. 2). As a result, sensitivity was decreased by 2%, but the specificity of the EIA kit was increased by 9%. Overall diagnostic accuracy was the same using either cut-off value (Table I).

Performance of Milenia H. pylori *IgG*

The manufacturer suggested a cut-off value of ≥44 EIA units for the Milenia EIA kit. This resulted in 8 false-negative and 10 false-positive patients. At this cut-off value, the sensitivity was 90% (83%–96%), the specificity 73% (55%–87%) and the overall diagnostic accuracy 86% (80%–92%) (Table I). ROC curve analysis (Fig. 1) showed that the optimal cut-off value was ≥51 EIA units. At this cut-off value, there were 13 false-negative and 7 false-positive patients (Fig. 3). As a result, the sensitivity was decreased by 4%, but specificity of the EIA kit was increased by 9%. Overall diagnostic accuracy was the same regardless of either cut-off value (Table I).

Comparison of performances between the two EIA kits

Comparison between the Pyloriset area under the ROC curve (0.86, 95% CI 0.79–0.92) and that of the Milenia (0.87, 95% CI 0.80–0.93) EIA kit showed no statistically significant difference ($P = 0.77$) (Fig. 1).

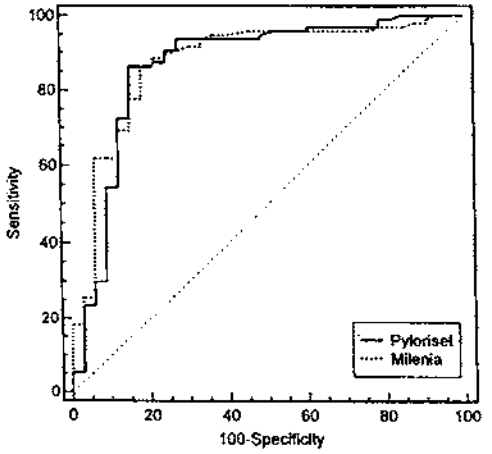

Fig. 1. Comparison of receiver operating characteristic (ROC) curves of two enzyme-linked immunosorbent assays for antibodies to *Helicobacter pylori*. The vertical axis shows true positive rate (sensitivity) and the horizontal axis false-positive rate (100 specificity). The ROC curve is plotted for different cut-off points. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. There is no statistically significant difference of the area under the receiver operating characteristic curve between the two assays ($P = 0.77$).

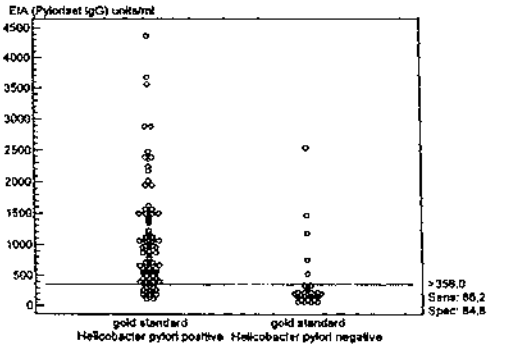

Fig. 2. Results of enzyme-linked immunosorbent assay (Pyloriset EIA-G, Orion Diagnostica, Espoo, Finland) for serum *Helicobacter pylori* IgG in 130 subjects according to *H. pylori* status. The optimal cut-off value of ≥358 units/ml was defined using the receiver operating characteristic curve analysis.

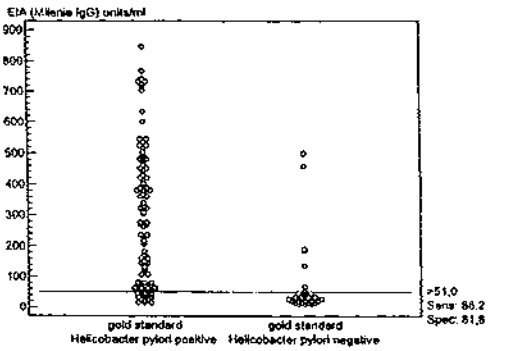

Fig. 3. Results of enzyme-linked immunosorbent assay (Milenia *H. pylori* IgG, DPC Bierman GmbH, Germany) for serum *Helicobacter pylori* IgG in 130 subjects according to *H. pylori* status. The optimal cut-off value of ≥51 units/ml was defined by using the receiver operating characteristic curve analysis.

Discussion

The diagnosis of *H. pylori* infection can be made by invasive tests, i.e. histology, rapid urease test and culture necessitating endoscopy and by non-invasive tests, i.e. IgG serology, ^{13}C-urea breath test and antigen stool detection. Current guidelines (7, 16) recommend therapy of *H. pylori* infection without endoscopy in symptomatic patients younger than 45 years without alarming symptoms. However, it should be mentioned that there is a lack of evidence supporting the clinical usefulness of treating *H. pylori* colonization in patients with functional dyspepsia and asymptomatic individuals. The Maastricht recommendations highlight the

Table I. Performance of enzyme-linked immunosorbent assay (EIA) kits for serodiagnosis of *Helicobacter pylori* infection in Greek patients. Comparison of the cut-off values as provided by the manufacturer and by the ROC curve analysis for the population studied. Results are presented with 95% confidence intervals (95% CI). Histology was used as gold standard, i.e. to define *H. pylori*-positive or negative patients.

Cut-off value (units/ml)	Sensitivity (%)	Specificity (%)	PPV[1] (%)	NPV[2] (%)	ODA[3] (%)
		Milenia IgG EIA			
≥44*	90 (83–96)	73 (55–87)	91 (86–96)	74 (66–81)	86 (80–92)
≥51**	86 (78–92)	82 (65–93)	93 (87–97)	68 (62–79)	86 (80–92)
		Pyloriset EIA			
≥300*	88 (80–94)	76 (58–89)	91 (86–96)	71 (61–77)	85 (79–91)
≥358**	86 (78–92)	85 (68–95)	94 (89–97)	69 (60–76)	85 (79–91)

[1]PPV = positive predictive value, [2]NPV = negative predictive value, [3]ODA = overall diagnostic accuracy, * cut-off value suggested by the manufacturer, ** cut-off value determined for the population studied by ROC curve analysis.

importance of the non-invasive tests for the detection of *H. pylori* infection. It has repeatedly been shown that most of the above tests have sensitivity, specificity and overall diagnostic accuracy higher than 90% (8, 17). However, IgG serology is the cheapest test. Besides, it is also readily available to general practitioners in most countries.

In the present study, we have prospectively evaluated the performance characteristics of two commercially available EIA kits (Pyloriset EIA-G, Orion Diagnostica, Espoo, Finland and Milenia *H. pylori* IgG, DPC Bierman GmbH, Germany) for the detection of *H. pylori* infection in our adult population. We have excluded patients with prior treatment for *H. pylori* and those who had taken proton-pump inhibitors, bismuth compounds or antibiotics within the previous 2 months. We can, therefore, reasonably assume that serology testing reflected active *H. pylori* infection in our patients. Furthermore, our gold standard was *H. pylori* identification on histology of four biopsies taken from the gastric antrum and body to reduce sampling error (18). It is known that an unequivocal single gold standard does not exist for *H. pylori* infection. However, histology with Warthin-Starry stain gives the best sensitivity (93%) and specificity (99%) (8) when compared with rapid urease tests (CLO test) (19) and urea breath test (19).

The accuracy of diagnostic testing by serology for *H. pylori* infection depends first on the sensitivity of the test, i.e. the percent of *H. pylori*-infected patients who are scored positive by the test and second on the specificity of the test, i.e. the percentage of negative patients who are correctly identified negative by the test. Furthermore, the overall diagnostic accuracy of the test depends on the ethnicity (12) and the prevalence (pre-test probability) of *H. pylori* infection in the community under investigation (13). For example, it has been shown that there are differences of test results between Belgians and those from Mediterranean areas (15). It has therefore been accepted that the cut-off values for an EIA kit should be tested and if necessary adjusted before use in certain communities (10). This is because cut-off values suggested by the manufacturer of the EIA kit may be different from the optimal cut-off values for the population under investigation.

The Pyloriset EIA-G kit has been evaluated in several studies. Laheij and co-authors (10) evaluated 15 studies published before 1998, and including a total of 1807 patients. In most of these studies the recommended cut-off value of 300 IU/ml was used and yielded a median sensitivity of 90% and specificity of 89% (10). However, a higher optimal cut-off value (445 IU/ml) was determined by ROC curve analysis by Meijer and co-authors (14) for the Dutch population, with a 50% prevalence of *H. pylori* infection. In our study, ROC curve analysis suggested that the optimal cut-off value be 358 IU/ml for the Greek population, with a 40% prevalence rate of *H. pylori* infection (20). This cut-off value reduced sensitivity by 2% but increased the specificity of the test by 9%.

Because of inherent differences in antibody responses, a different optimal cut-off might be obtained for sera of patients stratified on different diagnoses, e.g. duodenal ulcer, non-ulcer dyspepsia. Another factor which may influence the cut-offs and therefore the performance of a given serological test is the gold standard used in each published study. Since most studies use a variety of combinations of reference tests as gold standard, performances of an EIA kit and cut-off values may differ between studies.

A formal evaluation of the Milenia *H. pylori* IgG EIA kit has never been published. There are only two publications, one from Spain (in Spanish), which included 48 patients (21), and one from our group, which included 189 Greek patients (22). Both studies used the cut-off value recommended by the manufacturer of 44 IU/ml. In the Greek study, sensitivity of the kit was 94% but specificity was low (70%) (22). Sensitivity and specificity were even lower in the Spanish study (21). After adjusting the cut-off value from 44 IU/ml to that provided as optimal (51 IU/ml) by ROC curve analysis for our population investigated in the present study, the sensitivity of the EIA kit was reduced to 86%, but specificity was increased to 82%.

The higher the prevalence of *H. pylori* infection in the community, i.e. the pretest probability of the disease, the more accurate the serology (23). Besides the accuracy of a non-invasive test to diagnose *H. pylori* infection, other important issues are the availability and cost of the test. A recent comparison among the non-invasive tests has shown that serology is two to three times cheaper than the ^{13}C-urea breath test in the USA and Europe (6, 19). Serology is also less expensive than the detection of stool antigen. Another important use of serology is in diagnosing *H. pylori* infection in patients with atrophic corpus gastritis. Recent evidence suggests that histology and the ^{13}C-urea breath test may miss active *H. pylori* infection in a substantial number of these patients (24, 25).

In conclusion, our data show that both the Pyloriset and Milenia EIA kits performed equally well in detecting *H. pylori* infection in our patients. In addition, they show that before a serology kit is used in certain communities the optimal cut-off value should be determined by ROC curve analysis.

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Omeprazole triple therapy versus omeprazole quadruple therapy for healing duodenal ulcer and eradication of *Helicobacter pylori* infection: a 24-month follow-up study

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Objective To evaluate the efficacy of omeprazole triple therapy versus omeprazole quadruple therapy for *Helicobacter pylori* infection.

Design Prospective, randomized, single-centre, investigator-blind study.

Settings Departments of Gastroenterology and Histopathology, Evangelismos Hospital, Athens, Greece.

Methods One hundred and forty-nine consecutive patients with active duodenal ulcer were randomized to receive omeprazole (20 mg b.d.), amoxicillin (1 g b.d.) and clarithromycin (0.5 g b.d.) (OAC_{10}, $n = 78$), or omeprazole (20 mg b.d.), colloidal bismuth subcitrate (120 mg q.i.d.), metronidazole (0.5 g t.i.d.) and tetracycline hydrochloride (0.5 g q.i.d.) ($OBMT_{10}$, $n = 71$) for 10 days. Patients' symptoms were scored, and compliance and treatment-related side effects were assessed. Endoscopy was performed before treatment and at 10–12 weeks and 12 months after treatment. *H. pylori* infection and its successful eradication were sought by histology, immunohistochemistry and campylobacter-like organisms (CLO) tests on multiple biopsies taken from the gastric antrum, corpus and fundus. Patients were re-evaluated clinically and underwent a ^{13}C-urea breath test (UBT) at 21–24 months. Those with dyspepsia and/or recrudescence of *H. pylori* were re-endoscoped.

Results Patient groups were comparable for age, sex, smoking, occasional use of nonsteroidal anti-inflammatory drugs (NSAIDs), and current or past bleeding episodes. Six and seven patients in the OAC_{10} and $OBMT_{10}$ treatment groups, respectively, were lost to follow-up. Eight patients were non-compliant. Two ulcers in the OAC_{10} group and one in the $OBMT_{10}$ group did not heal. By intention-to-treat (ITT) and per-protocol (PP) analyses, ulcer healing rates were 86% (67/78) and 97% (67/69), respectively, for the OAC_{10} group, and 82% (58/71) and 98% (58/59), respectively, for the $OBMT_{10}$ group. *H. pylori* eradication at 10–12 weeks after treatment was 78% (61/78) and 88% (61/69) for OAC_{10}, and 65% (46/71) and 78% (46/59) for $OBMT_{10}$, by ITT and PP analyses, respectively ($P > 0.1$). Side effects were more common with $OBMT_{10}$. Relapse rates of *H. pylori* were 3% and 2% for the first and second years, respectively. Four *H. pylori*-negative patients developed reflux symptoms, but only two developed erosive oesophagitis between 12 and 24 months.

Conclusions OAC_{10} and $OBMT_{10}$ were equally effective in healing active duodenal ulcers and eradicating *H. pylori*, but OAC_{10} should be used as a first-line treatment because of its better tolerance. *Eur J Gastroenterol Hepatol* 14:1237–1243 © 2002 Lippincott Williams & Wilkins

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Keywords: omeprazole, clarithromycin, amoxicillin, bismuth triple therapy, quadruple therapy, duodenal ulcer, *Helicobacter pylori*

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Introduction

The optimal therapy for *Helicobacter pylori* infection remains uncertain. The pioneer combination of bismuth, metronidazole and tetracycline hydrochloride (BMT, or 'classical' triple therapy) was effective [1–4], but poor compliance and resistance to metronidazole [5,6] led to its replacement by proton pump inhibitor (PPI)-based triple therapies [7–10]. However, in the clinical setting, PPI triple therapies achieve eradication rates ranging from 70 to 87% [11]. When omeprazole was shown to improve the results of BMT treatment by enhancing the efficacy and lessening side effects and duration of treatment [12,13], the combination of omeprazole, bismuth, metronidazole and tetracycline (OBMT) was proposed as the gold standard for the treatment of *H. pylori* infection [14–17]. However, other investigators consider OBMT to be a 'rescue therapy' when other regimens have failed [18–22]. In

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fact, a recent meta-analysis of 24 studies showed comparable eradication rates of *H. pylori* for PPI quadruple and PPI triple therapies [11]. However, the comparative efficacy and compliance of any of the PPI triple and quadruple therapies have not been studied adequately in prospective clinical trials. Thus, the aim of this prospective, randomized, investigator-blind, single-centre trial was to evaluate the efficacy of omeprazole, amoxicillin and clarithromycin given for 10 days (OAC_{10}) and $OBMT_{10}$ in healing duodenal ulcer and eradicating *H. pylori* infection. In addition, the study aimed to assess in the long-term the post-eradication relapse rates of *H. pylori* infection and patient outcome.

Methods

Between January 1995 and December 1998, 162 consecutive patients with an endoscopically confirmed active duodenal ulcer were evaluated for this prospective, randomized, investigator-blind, single-centre study. Some patients had experienced at least one bleeding episode from duodenal ulcer in the past. Ethical approval was obtained. All patients gave written consent before study entry. Exclusion criteria were chronic alcoholism, chronic renal or hepatic failure, malignant disease, previous gastric surgery, treatment with anticoagulants, treatment with antibiotics during the month preceding study entry, and well-documented allergy to any of the study drugs.

Endoscopy (Olympus GIF Q10) was performed on an outpatient basis. Any lesions found were graded for severity [4], and biopsies were taken from relatively uninvolved mucosa (away from areas of gross inflammation or erosions) of the antrum ($n = 4$) and body/fundus ($n = 5$) of the stomach. Two biopsy specimens (one from the antrum and one from the body) were processed immediately for a rapid urease test (campylobacter-like organisms test, CLO test, Delta West Ltd, Bentley, Australia). The remaining seven biopsy specimens were oriented properly, fixed in 10% formalin, embedded in paraffin, and processed for histology and immunohistochemistry.

Patients with an active duodenal ulcer and a positive CLO test were randomized to receive one of the following treatments: (1) omeprazole (20 mg twice daily before meals), amoxicillin (1 g twice daily with meals) and clarithromycin (500 mg twice daily with meals) (OAC_{10}) or (2) omeprazole (as previously), bismuth subcitrate (CBS, De-Nol, one swallowable tablet containing 120 mg bismuth four times a day, before meals and at night 2 h after dinner), metronidazole (500 mg three times a day before meals) and tetracycline hydrochloride (500 mg tablets four times a day, before meals and at bedtime) ($OBMT_{10}$). All patients received omeprazole (20 mg twice daily) for an additional 4 days. Any acid-suppressive drugs were stopped at least 3 days before study entry and were not allowed during the entire study period. Although patients were randomized in the study on the basis of a positive CLO test at the index endoscopy, they were continued in the study only when histology confirmed *H. pylori* infection. Patients not fulfilling this criterion were withdrawn from the study.

Before and after treatment, patients rated their general wellbeing on a visual analogue scale (VAS); their symptoms were also assessed and scored by a single physician [4]. Patients were seen in the outpatient clinic when they completed the therapy. Compliance with treatment was assessed by counting the returned tablets of each study medication. Treatment-related side effects were assessed by a questionnaire form. Finally, a further appointment for endoscopy was arranged.

Repeat endoscopy was performed 10–12 weeks after completion of therapy. Biopsies were obtained using the same protocol. To assess the long-term effects of treatment, patients were asked to return for an annual follow-up endoscopy with gastric biopsies. *H. pylori* was sought on gastric biopsies by CLO tests, histology (haematoxylin and eosin and modified Giemsa) and immunohistochemistry (rabbit anti-*H. pylori* monoclonal antibody, Dako). Severity and activity of gastritis were also scored before and after treatment using the Houston update of the Sydney classification [23]. Cure was defined as the absence of *H. pylori* infection by CLO tests, histology and immunohistochemistry.

H. pylori-negative patients at 12 months were asked to undergo a ^{13}C-urea breath test (UBT) and consult the outpatient clinic after 1 year or whenever they developed dyspeptic or reflux symptoms. Symptomatic patients, irrespective of their *H. pylori* status, as well as patients with a positive UBT, underwent endoscopy using the same biopsy protocol.

Endoscopies and CLO tests were performed by a single physician (GJM) unaware of the patient's treatment category. A physician unaware of the patient's history and endoscopy performed randomization, assessment of symptoms and assessment of compliance with treatment. Histology was assessed by an experienced gastrointestinal pathologist (KP) unaware of the patient's status or treatment category.

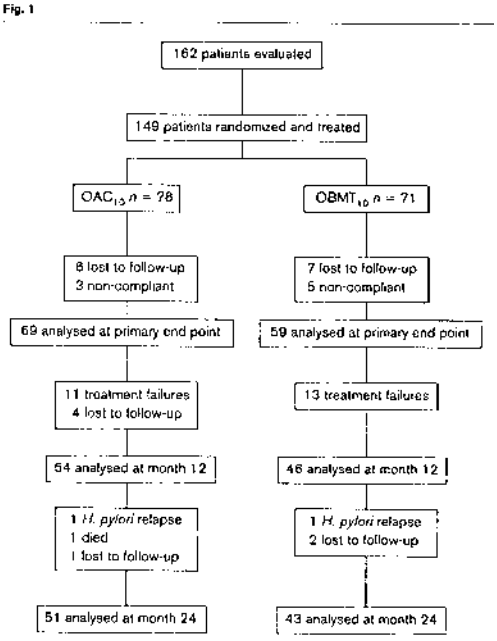
Statistical analysis

The primary end points of this study were endoscopic healing rates of duodenal ulcers and eradication of *H. pylori* 10–12 weeks after therapy. Secondary end points were the influence of demographic and clinical parameters on eradication of *H. pylori*, relapse rate of *H.*

pylori infection and duodenal ulcer after 12 and 24 months, and de novo development of post-eradication reflux oesophagitis.

The results of treatment were analysed by the intention-to-treat (ITT) and per-protocol (PP) methods. The former included only patients with confirmed evidence of *H. pylori* infection and duodenal ulcer before treatment but also included as treatment failure patients who did not return for re-evaluation after treatment. The latter included only patients who were eligible for evaluation at each specific visit. Comparisons between the two treatment groups were made using the Mann–Whitney U test and the chi-squared test or the Fisher's exact test, where appropriate. Exact binomial 95% confidence intervals were calculated for *H. pylori* eradication. Multivariate logistic regression analysis was performed to evaluate the effect of potential risk factors on the outcome of treatment, including age, gender, smoking (yes/no), bleeding ulcer (yes/no), ulcer size (< 1 cm, > 1 cm), disease duration, gastritis score (severity and activity) in the antrum and corpus of the stomach before eradication, alcohol use (social drinking/no), and occasional nonsteroidal anti-inflammatory drug (NSAID) use (occasional/no). Elaboration of data was accomplished by the Statgraphics Statistical Package (SSP). P values of less than 5% (two-sided) were considered significant.

Fig. 1



Flowchart of patients and reasons for discontinuation.

Results

Overall, 162 patients were evaluated for the study, but 149 patients were finally randomized to receive OAC_{10} ($n = 78$) or $OBMT_{10}$ ($n = 71$) (Fig. 1). Six and seven patients in the OAC_{10} and $OBMT_{10}$ groups, respectively, felt well and did not return for endoscopy at week 10. Three and five patients in the OAC_{10} and $OBMT_{10}$ groups, respectively, were non-compliant (< 95% of drugs taken). Thus, PP analysis was based on 69 patients in the OAC_{10} group and 59 patients in the $OBMT_{10}$ group. The demographic and clinical characteristics of patients at randomization (ITT analysis) and of patients completing the trial (PP analysis) are given in Table 1. There were no significant differences in any patient- or disease-related parameters between treatment groups at study entry or study termination.

At entry, all diagnostic tests for *H. pylori* (CLO test, histology, immunohistochemistry) were 100% concordant.

Comparison of VAS scores between the OAC_{10} and $OBMT_{10}$ groups before and after treatment was not significantly different. However, there was a significant improvement between post- and pretreatment VAS scores within each group. The median value of the VAS score fell from 85% to 15% in the OAC_{10} group

Table 1 **Patient demographics and clinical characteristics***

	Intention to treat		Per protocol	
	OAC_{10} ($n = 78$)	$OBMT_{10}$ ($n = 71$)	OAC_{10} ($n = 69$)	$OBMT_{10}$ ($n = 59$)
Age (years) (mean, range)	40 (16–69)	40.5 (17–70)	39 (16–69)	40.5 (17–70)
Sex (M/F)	40/38	35/36	35/34	29/30
Disease duration (years) (mean, range)	4.1 (1–17)	5.0 (1–19)	4.0 (1–17)	5.0 (1–19)
Recent/past bleeders	20	22	20	22
Smokers	54	52	48	43
Social drinkers	30	28	25	24
Occasional NSAID users	33	29	27	23

*Differences between groups were not significant.
NSAID, nonsteroidal anti-inflammatory drug.

($P < 0.001$) and from 82% to 20% in the $OBMT_{10}$ group ($P < 0.0001$).

Endoscopy at 10–12 weeks after therapy revealed that all except three duodenal ulcers had healed. Two patients had received OAC_{10} and one $OBMT_{10}$ without eradication of the *H. pylori* (Table 2). At index endoscopy, two patients had large (> 20 mm) ulcers and one patient had kissing ulcers and severe duodenitis; they were heavy smokers (> 30 cigarettes/day).

The ITT and PP rates for ulcer healing and eradication of *H. pylori* are given in Table 2. There were no significant differences in ulcer healing rates between OAC_{10} and $OBMT_{10}$. Although OAC_{10} was superior to $OBMT_{10}$ in eradicating *H. pylori*, differences were not significant. Multiple logistic regression analysis revealed that smoking was the only independent factor related significantly to eradication failure (Table 3). When corrected for demographic and clinical confounding factors, the statistical analysis revealed no superiority of any regimen in eradicating *H. pylori* in bleeders, smokers or occasional NSAID users. Eradication rates for occasional NSAID users were 76% (25/33) and 85% (23/27) in the OAC_{10} group, and 65% (19/29) and 77% (18/23) in the $OBMT_{10}$ group (ITT and PP analyses, respectively). Eradication rates for NSAID non-users were 80% (36/45) and 90% (38/42) in the OAC_{10} group and 67% (28/42) and 78% (28/36) in the $OBMT_{10}$ group (ITT and PP analyses, respectively). There were no significant differences by ITT or PP analysis regarding eradication rates in NSAID users or non-users between the two arms of the study or between users and non-users in each arm of the study.

Eight (4.9%) patients were non-compliant (< 95% of drugs taken). Three had received OAC_{10} but stopped treatment before the fifth day because of nausea, headache, and diarrhoea, respectively. Five patients had received $OBMT_{10}$ but stopped treatment before the fourth day because of nausea (3), metallic taste (2), headache (1), vomiting (1) and dizziness (1). Differences in compliance between groups were not significant. In general, $OBMT_{10}$ was associated with a much higher incidence of side effects than OAC_{10} ($P < 0.01$), but these were mostly minor and easily tolerated, did not interfere with daily activities, and did not prevent patients from completing the trial.

Table 3 Multivariate logistic regression analysis of independent risk factors for failure to eradicate *Helicobacter pylori*

	OR	95% CI	*P* value
Gender	0.80	0.30–2.05	0.64
Age	0.75	0.27–2.08	0.59
Bleeding ulcer	2.13	0.26–5.38	0.08
Ulcer size	1.09	0.49–2.89	0.99
Disease duration	0.80	0.19–1.02	0.61
Use of NSAIDs	1.01	0.37–2.77	0.98
Social drinking	1.10	0.25–4.80	1.05
Current smoking	3.75	1.40–6.10	0.01
Gastritis (antrum)	0.95	0.40–1.79	0.60
Gastritis (corpus)	0.85	0.30–1.85	0.68

CI, confidence interval; NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio.

Negative CLO tests but positive histology and immunocytochemistry for *H. pylori* were found in three patients. The density of *H. pylori* population was graded as approximately 1 (range 0–3). These patients were considered as treatment failures. Indeed, 8–10 months after treatment, these patients developed dyspepsia. Follow-up endoscopy revealed relapse of duodenal ulcer; the CLO tests were positive and the density of *H. pylori* population was 2–3.

Annual follow-up endoscopy was performed in 100 patients who were *H. pylori* negative at weeks 10–12 after treatment (54/61 patients in the OAC_{10} group and 46/46 patients in the $OBMT_{10}$ group; see Fig. 1). Two of these patients, one in each arm of the study, were *H. pylori* negative, but they developed heartburn and at endoscopy grade I erosive oesophagitis was found. Ninety-eight patients had remained entirely asymptomatic throughout this period, and endoscopy was negative. However, *H. pylori* infection relapsed in three patients (one in the OAC_{10} group and two in the $OBMT_{10}$ group); the annual relapse rate was 3%.

Eradication failures as well as non-compliant patients were followed up in the outpatient clinic. When dyspeptic symptoms recurred, these patients underwent

Table 2 Ulcer healing and *Helicobacter pylori* infection eradication rates

	Healing of duodenal ulcer (*n*, %, 95% CI)		*H. pylori* eradication rate (*n*, %, 95% CI)	
Treatment group	ITT	PP	ITT	PP
OAC_{10}	67/78 (86%) (78–94%)	67/69 (97%) (93.0–101.0%)	61/78 (78%) (68–88%)	61/69 (88%) (80–96%)
$OBMT_{10}$	56/71 (82%) (81–97%)	56/59 (98%) (94–102%)	46/71 (65%) (47–83%)	46/59 (78%) (77–79%)
P value	0.8	0.9	0.1	0.2

CI, confidence interval; ITT, intention to treat; PP, per protocol.

endoscopy that revealed a relapse of ulcer and the presence of *H. pylori*. All relapses occurred 8–11 months after treatment failure.

Ninety-four *H. pylori* negative patients were re-evaluated between 23 and 25 months post-treatment (Fig. 1). Three patients were lost to follow-up and one had died. Two patients were found positive by UBT. Endoscopy was negative but histology confirmed the relapse of infection. Another two *H. pylori*-negative patients developed endoscopy-negative reflux symptoms. Thus, the overall relapse rate of *H. pylori* at 2 years was 5%. During this period, 4% of the patients developed reflux symptoms, but only two of these had mild erosive oesophagitis.

Discussion

In this prospective, randomized, investigator-blind, single centre study performed in unselected, consecutive Greek patients with active duodenal ulcer, $OBMT_{10}$ was not superior to OAC_{10} in healing duodenal ulcers or eradicating and preventing recrudescence of *H. pylori* infection. In fact, there was a 10% therapeutic gain for OAC_{10} over $OBMT_{10}$. These results are in accordance with recent studies [18–22] and a large meta-analysis [11], which have shown comparable eradication rates of omeprazole-based triple and quadruple therapies. Similar results have been reported recently in trials of lansoprazole-based triple and quadruple therapies [24,25]. In addition to therapeutic efficacy, compliance was much better with the OAC_{10} regimen. More patients on $OBMT_{10}$ were withdrawn from the study due to treatment-related side effects, and significantly more patients developed minor adverse events not leading on to trial discontinuation. Again, this was similar to reports for lansoprazole-based triple and quadruple therapies [24,25], although compliance of BMT can undoubtedly be improved with a new all-in-one formulation [26]. These results suggest that OBMT should be reserved as a 'rescue' rather than a first-line therapy for the eradication of *H. pylori* infection.

Various factors, such as antibiotic resistance, smoking, and duration of treatment, may account for the discrepant performance of OBMT in this and other European trials. In the $OBMT_{10}$ group, metronidazole resistance might be responsible for the relatively low eradication rates of *H. pylori* infection. Unfortunately, we are unable to produce data on pretreatment susceptibility of *H. pylori* because this study was performed before methods of culturing *H. pylori* were available in our hospital. However, the prevalence of *H. pylori* metronidazole resistance in Greece was reported to be 46% in 1991 [27]. The primary resistance of *H. pylori* strains isolated from Greek patients in the ACT-10 [10] study was 54%. More recently, in a European collaborative study of 22 centres from 17 European countries, the primary resistance of *H. pylori* strains isolated from Greek patients never treated with metronidazole or clarithromycin was 47% compared with an average of 33% in other centres (Dr A. Mentis, Pasteur Institute of Athens, personal communication). This figure was the highest among Western European countries participating in the study and one of the highest in Europe. Assuming that roughly 50% of our patients carried metronidazole-resistant strains, $OBMT_{10}$ has overcome to a certain degree metronidazole resistance. Even so, we did not observe the complete overcome of metronidazole resistance by omeprazole-based quadruple therapies reported in European studies [16,17]. Our results are similar to those of Phull *et al.* [28], who reported eradication rates of 65%, 60% and 60% by BMT, O(20 mg)BMT and O(40 mg)BMT, respectively. This is an important reason why OBMT should not be used as a first-line therapy in countries with a high prevalence of metronidazole-resistant strains of *H. pylori*. The use of OBMT as first-line therapy for *H. pylori* infection is probably justified only for patients harbouring *H. pylori* strains sensitive to metronidazole.

Clarithromycin resistance may also have influenced eradication rates of *H. pylori* infection in the OAC_{10} arm of our study. Between 1991 and 1995, no primary resistance of *H. pylori* to clarithromycin had been detected in adult Greek patients, although 5% of dyspeptic children harboured clarithromycin-resistant strains [10]. However, clarithromycin resistance was recently 10% compared with an average of 10.5% in Europe (Mentis, personal communication).

Duration of treatment may influence eradication rates. The choice of a 10-day OAC regimen was based on allegations that a 14-day regimen may be longer but a 7-day course shorter than needed. It seems that this issue has now resolved in Europe, although 10-day regimens prevail in the USA. However, it has been reported recently that prolonging OAC treatment from 6 to 12 days in patients with non-ulcer dyspepsia increases the therapeutic gain by 21% [29]. OBMT was given for 10 days because of reports claiming fewer side effects and improved compliance without compromising efficacy [12]. Indeed, while this work was in progress, de Boer *et al.* [14–17] reported that even a 7-day OBMT regimen identical to ours may cure more than 90% of patients carrying metronidazole-resistant strains. The same group has suggested that lansoprazole-based quadruple therapies maintain efficacy even if their duration is reduced to 4 days [30] or even 1 day [31]. However, eradication rates achieved by a 7-day OBMT in patients with bleeding ulcers were significantly higher than those achieved by a 2-day OBMT [32]. It is possible, therefore, that shortening the duration of OBMT may compromise efficacy in a population with a high prevalence of metronidazole-resistant *H. pylori* strains.

Although smoking reduces *H. pylori* eradication rates in triple therapies [4,33–35], the reduction in eradication rates of *H. pylori* infection was equal in both arms of this study. This study is also in agreement with Kamada *et al.* [33] that severity of gastritis may influence the outcome of *H. pylori* treatment.

Recrudescence of *H. pylori* infection at 12 and 24 months lay within the expected range [35,36]. Furthermore, the rate of de novo development of post-eradication oesophagitis was also very low. No single factor could account for the development of oesophagitis, because all these patients had moderate to severe corpus gastritis before treatment and adopted a liberal lifestyle in the post-eradication era by resuming smoking and gaining significant body weight.

In conclusion, OAC_{10} and $OBMT_{10}$ were equally effective in healing duodenal ulcers and eradicating *H. pylori*. However, $OBMT_{10}$ did not achieve its attributed very high eradication rates probably because of metronidazole resistance, duration of treatment, or any other confounding factor. Based on evidence, PPI triple therapies should be preferred in areas with a high prevalence of metronidazole resistance, and OBMT should be reserved as a 'rescue' therapy for patients failing to eradicate *H. pylori* on a PPI triple therapy.

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Original Communication

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Helicobacter pylori Infection in Patients Undergoing Appendectomy

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Summary

Aims: Helicobacter pylori has been found in the upper gastrointestinal tract; it is incriminated as aetiological factor in various pathological conditions. This prospective study assesses the presence of this microorganism in the appendix flora and the possible role of its infection in the pathogenesis of acute appendicitis.

Methods: H. pylori was investigated in 46 consecutive patients undergoing emergent appendectomy for presumed acute appendicitis. Blood sample for serological test of H. pylori infection was drawn before operation. The removed appendix specimen was stained for H. pylori; confirmation was made by PCR (Polymerase Chain Reaction) analysis. The intensity of inflammation was determined pathologically grading from no inflammation to gangrenous appendicitis. Statistical analysis was made using the chi-square test.

Results: Seropositivity for H. pylori infection was found in 18 patients (39%), but the microbe was detected in just two appendix specimens (4%). In all seropositive patients acute appendicitis was confirmed by the pathology study; serous (33%) and purulent or gangrenous (67%). The latter incidence in the seronegative patients was 50%. There were found eight specimens (17%) negative for inflammation dealing all with seronegative patients.

Conclusions: It seems that H. pylori colonizes the appendix in small proportion and is unlikely to be associated in direct correlation with acute appendicitis. However, seropositive patients with acute inflammation are likely to suffer from purulent or gangrenous form.

Keywords: Helicobacter pylori infection, appendix, acute appendicitis

Introduction

The culture of Helicobacter pylori and its recognition as spiral bacterium of the genus Campylobacter has been achieved in 1982 [1]. It is a Gram-negative organism that is related in high incidence to low socioeconomic conditions and is associated with various gastro-intestinal diseases [2]. The oral cavity has been implicated as a reservoir of its systemic infection [3], but this has not been confirmed as important factor [4]. Although, it is the commonest chronic infection, nowadays, the exact way of transmission is unknown. It has been isolated from human faeces and the fecal-oral sequence might be a route of infection [5].

The stomach is the frequent site of H. pylori colonization. An infection by this organism has been found in almost all patients with active chronic gastritis, in up to 90 per cent of those with duodenal ulcer and about 70 per cent of those with gastric ulcer [6, 7]. Furthermore, it is associated with an increased risk of gastric adenocarcinoma considered as contributing factor [8]; its eradication could reduce the incidence of gastric cancer [9]. H. pylori has been found

in association with MALT (mucosa-associated lymphoid tissue) lymphoma of the stomach [10] and rectum [11]; a regression of the lesion has followed the successful eradication with antibiotic therapy.

There is limited knowledge about H. pylori existence outside the gastro-duodenal region. The presence within the appendix and its possible role in the aetiology of acute appendicitis has not been investigated adequately. There are very few studies on this topic [12–14]. This prospective trial was designed to contribute further by confirming or not these results.

Patients and Methods

This study includes 46 consecutive patients, in whom an emergent appendectomy for presumed acute appendicitis was performed in our department over a period of six months (the second half of 2000). There were 24 women (52%) and 22 men (48%) with mean age of 49 years (range 16 to 72 yr.). None of them had a history of previous H. pylori known infection or eradication therapy. They suffered from a right iliac fossa pain and had the clinical signs of acute inflammation. The details of all patients underwent appendectomy in our department over the past twenty years were retrieved from an electronic database. They include a total of 1546 patients (women 51%, mean age of 51 with range 15–87 years), who suffered from a right iliac fossa pain and had an emergent operation for presumed acute appendicitis.

Blood sample for serological test of H. pylori infection was drawn before operation. An enzyme linked immunosorbent assay (ELISA) was performed (Kit, H. pylori-CHECK-1, MEGA-LAB, France) using a combination of human anti-IgG with high purity proteins of H. pylori for the detection of H. pylori IgG antibodies.

The removed appendix specimen was sent for histopathologic examination in every case asking the assessment of inflammation; it was stained (Giemsa stain) for H. pylori detection. The intensity of inflammation was graded from 0 to 3 (0 – no inflammation, 1 = serous, 2 = purulent, 3 = gangrenous or perforation).

The presence of H. pylori in the appendix specimen was regarded as positive after microscopic recognition and confirmation by PCR analysis. Statistical analysis was made using the chi-square test. Statistically significant was regarded when $p < 0.05$.

Results

The demographic characteristics of 46 studied patients did not differ with those of 1546 patients undergoing appendectomy for presumed acute appendicitis.

The histopathologic study of the removed specimen showed negative results for inflammation in eight cases (17%), serous inflammation in 12 cases (26%), purulent inflammation in 17 cases (37%) and necrotic inflammation in nine cases (19.5%). None other pathology was revealed in these cases.

The above results did not differ significantly with those found in the total of 1546 patients ($p > 0.05$). The latter included negative results for inflammation (19%), serous inflammation (30%), purulent inflammation (34%) and necrotic inflammation (17%).

The serology of the blood sample was positive for H. pylori infection in 18 out of 46 patients (39%). In all of them acute inflammation of the appendix was found in the pathology (serous 33%, purulent 39%, gangrenous 28%).

On the other hand, all specimens negative for inflammation (8 cases) belonged to the seronegative patients. In the seronegative patients the pathology was different of that above mentioned in the seropositive patients (no inflammation 28.5%, serous 21%, purulent 36%, gangrenous 14%). The differences between seropositive and seronegative patients were statistically significant. No inflammation 0 vs 28.5% ($p < 0.01$), purulent and gangrenous inflammation 67% vs 50% ($p < 0.05$).

All these results of H. pylori serology and histopathologic grace of appendicitis are combined completely in Table I.

The microscopic investigation in combination with the PCR analysis confirmed the presence of H. pylori in the appendix specimen in two cases (4%). Both cases were dealing with gangrenous inflammation of the appendix and positive serology of the blood sample.

Discussion

H. pylori IgG antibodies were detected by the applied serology of the blood sample in 39% of the included in this trial otherwise healthy patients. Based on the unselected consecutive patients, despite the small number of them, we could postulate that this percentage might reflect the seropositive rate in the healthy population. Likewise, in another study from a European country, the mean H. pylori prevalence was 51%. However, it was increased with age from 23% in young people with age of 20–29 years to 68% in those up to 70 years [2].

The accordance of both demographic characteristics and histopathologic results of 46 studied patients with those in the total of 1546 patients indicates reliable study cohort. In this trial the percentage of negative for inflammation histopathologic findings after appendectomy for presumed acute appendicitis rises to 17%; this is in absolute agreement with other trials, in which it is ranged from 15% to 25% [15–17].

Acute appendicitis was confirmed in all of our seropositive patients; all negative cases belonged to the seronegative patients.

Table I Combination of H. pylori serology with histopathologic grade of the appendiceal inflammation. The values in parenthesis are per cent.

H. pylori serology	Histopathologic grade				
	No inflammation	Serous	Purulent	Gangrenous	Total
Positive	0	6 (33)	7 (39)	5 (28)	18 (39)
Negative	8 (28.5)	6 (21)	10 (36)	4 (14)	28 (61)
Total	8 (17)	12 (26)	17 (37)	9 (19.5)	46 (100)

In addition, the incidence of purulent and gangrenous inflammation, which indicates the severity of the condition, was significantly higher in the seropositive than in the seronegative patients (67% vs 50%, $p < 0.05$). There is no reasonable explanation for these remarkable notes, since all cases were not accompanied by H. pylori colonization in the appendix. The latter was documented in just two patients (4%); they were seropositive and had gangrenous inflammation. This could be explained by the lymphoid tissue hypertrophy caused by the chronic stimulus of H. pylori infection. Furthermore, the above possible pathogenetic mechanism has been proposed for the development of MALT lymphoma [10, 11].

Two other trials have failed to confirm the presence of H. pylori in the appendix and its association with acute appendicitis [12, 13].

Histology may reveal organisms with morphological appearance like H. pylori, but PCR analysis is considered the "gold standard" that could identify the specific H. pylori strain confirming the diagnosis [13, 14].

In conclusion, we would say that this study has revealed presence of H. pylori in the appendix specimens. Although, this colonization is not implicated directly in the aetiology and pathogenesis of acute appendicitis, its presence could result more severe inflammation.

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Helicobacter pylori infection in pediatrics

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ABSTRACT

In the past year the main interest was focused on the role of family for transmission of *Helicobacter pylori* to children; the evaluation of noninvasive diagnostic tests, especially in young children; extra-intestinal clinical manifestations; the lack of consensus on treatment; and the problem of high resistance of the microorganism to antibiotics.

Introduction

Many papers have been published for *Helicobacter pylori* infection in childhood regarding epidemiology, mode of transmission, possible protection by breast milk, its relation with clinical symptoms, the reliability of noninvasive diagnostic techniques in children, and the problem of antibiotic resistance.

Epidemiology

Although *H. pylori* infection is common worldwide, the time of acquisition is unclear. Malaty et al. [1] investigated this issue in a cohort of children selected retrospectively from a population followed up for 21 years. *H. pylori* was detected serologically in 8% of the children aged 1–3 years. By age 18–23 years, the prevalence of infection was 24.5%. The median age of seroconversion was 7.7 years The rate of seroconversion per year was 1.1% and was higher among children aged 4–5 years (2.2% vs. 0.2% at age 18–19 years).

However, it has been difficult to assess accurately the route of transmission. Herbarth et al. [2] investigated, by ^{13}C urea breath test, a total of 3347 school beginners in Germany, and the *H. pylori* prevalence was 6.5%. Among urban children, the risk was significantly associated with contact to pet hamsters and travel to Asian countries. Among rural children, *H. pylori* positivity increased significantly with the following risk factors: drinking water from nonmunicipal sources, more than three children living in a household, and contact with pet hamsters.

Tindberg et al. [3] in a cross-sectional study, found that 16% of children aged 10–12 years were infected. The seroprevalence was 2% among children of Scandinavian parents and 55% among children born from parents originating from high prevalence areas. Infection among classmates was not a risk factor for *H. pylori* infection. They conclude that intrafamilial transmission is far more important than child-to-child transmission outside the family. The *H. pylori* prevalence in the parental generation may be a crucial determinant for the child's risk of contracting the infection. Wizla-Derambure et al. [4] also conclude that the source of *H. pylori* infection is intrafamilial rather than from a community, such as nursery or school attended at a young age. A high risk of intrafamilial infection was also shown by Taneike et al. [5].

To the contrary, in a recent study, Malaty et al. [6] highlight the importance of factors outside of the home for transmission. Children living in the most crowded day-care centers were at greater risk for *H. pylori* acquisition. In addition, breast feeding played a protective role against the acquisition of *H. pylori* infection.

In a Japanese study, Okuda et al. [7] also confirmed the protective role of breast feeding on *H. pylori* infection in early childhood. The mean period of breast feeding of those with a HpSA positive test was 5.3 months, while for the negative group it was 7.8 months ($p = .02$). They speculate that breast feeding offers some form of natural protection from *H. pylori* infection in early childhood, probably due to high levels of lactoferrin in the human milk. A low prevalence

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of infection in children, who were fed with breast milk containing high levels of anti-*H. pylori* antibodies has also been reported [8.]

Kitagawa et al. [9] investigated the possibility of maternal transmission of *H. pylori* in the perinatal period. They conclude that vertical transmission during pregnancy or delivery is unlikely as a route of mother-to-child transmission of *H. pylori*, while horizontal infection through breast feeding may occur.

Clinical manifestations

It is clear now that *H. pylori* infection is related with most upper gastrointestinal disorders in children. In a retrospective study of a cohort of 2550 symptomatic children, who underwent endoscopy over a 9-year period, primary peptic ulcer disease was diagnosed in 2% of children and *H. pylori* was identified in 54% of children with peptic ulcer. The prevalence of infection was higher in children with duodenal ulcer (60%) compared to those with gastric ulcer (20%, $p < .001$) [10].

The role of *H. pylori* remains unclear in children with recurrent abdominal pain (RAP) and has been one of the most debated topics. Reliable studies are hard to find. Ozen et al. [11] found that 60.3% of children with RAP were *H. pylori* positive and symptoms disappeared in 87% of children after eradication compared to 41% of those in whom infection was not cured. Uc and Chong [12] reported an improvement of dyspeptic symptoms in 16 *H. pylori* infected children with gastritis, but results are subject to criticism [13]. On the contrary, Wewer et al. in a double-blind treatment study of *H. pylori* infected children and RAP did not provide evidence for a causal relationship between RAP and *H. pylori* [14].

H. pylori is the major cause of antral gastritis and duodenal ulcer in children and is considered as risk factor of primary gastric MALT lymphoma [15]. Studies report that this microorganism is not only a gastric pathogen, but causes extra-intestinal manifestations in children such as unexplained iron deficiency anemia, even in the absence of gastrointestinal bleeding [16–18]. It has also been considered as an etiological agent of unexplained short stature and growth delay [16,19,20]. Dental caries were more common among *H. pylori*-positive children but a causal relationship between *H. pylori* and dental caries is unlikely [21].

Diagnosis

The indications for detection and the recommended diagnostic methods have been proposed in a consensus organized by a European Pediatric Task Force on *H. pylori* as well as in the statement of the NASPGHN [22,23]. Concern has been raised on the validation of noninvasive tests for assessing *H. pylori* infection in children, since most validated studies in children included only a small number of patients. Accuracy of most tests is lower in young children if the cut-off values used are the same as those established for older children or adults [24].

Invasive tests

Invasive tests diagnose both infection and disease, are invaluable for excluding severe gastroduodenal pathology and remain the 'gold standard'.

Luzza et al. [25] found antral nodularity only in the children infected with *H. pylori* (40.5% vs. 0%) with a specificity of 100% and sensitivity of 40%. They conclude that the endoscopic pattern of antral nodularity identifies children with *H. pylori* infection, severe gastritis and increased lymphoid follicles. Antral nodularity and positive CagA serology could be considered as even more relevant markers of severe gastric inflammation in children with *H. pylori* infection [26].

Antigastric autoantibodies (AGA) [27] as well as proinflammatory cytokines [28] were detected in significantly higher concentrations in the gastric mucosa of *H. pylori*-positive than *H. pylori*-negative children.

Eshun et al. [29] compared immunohistochemical and silver stains of pediatric biopsy sections for the identification of *H. pylori* infection with chronic inflammation and a negative urease screening test (CLO®). They recommend the use of immunohistochemical staining rather than silver staining in the evaluation of urease negative gastric biopsies demonstrating chronic inflammation in children.

Noninvasive tests

Urea breath test

The ^{13}C-UBT is a reliable method and has been used for the assessment of *H. pylori* gastric colonization and post-eradication therapy in older children, with a high specificity and sensitivity. However, this test needs a validation in children less than 2 years old.

Imrie et al. [30] studied 72 children less than 4 years old. Results could not be analyzed in 12.5% of the children because of inadequate breath samples, indicating that the test is technically more difficult to perform in young children. In children older than 2 years, the specificity and positive predictive value (PPV) of the test was 96% and 80%, respectively, compared with the gold standard invasive tests. However, values decreased to 91% and 50% in children less than 2 years old.

Serology

Serological testing to diagnose *H. pylori* infection in children is still controversial, although commonly used in clinical practice. Because of the controversial results obtained with children, the consensus statement previously mentioned considered serological testing to be less reliable in children than in adults, but further validation studies and improvement of tests are warranted [22].

Kinderman et al. [31] evaluated two commercial enzyme immunoassays (EIAs), testing IgG and IgA responses for diagnosis of *H. pylori* infection in children. They conclude that the specificity of commercial serological tests is high in children when the cut-off values obtained from adults are used. In contrast, sensitivity is variable, with a strong age dependence in some, but not all of the tests.

Tindberg et al. [32] also evaluated the accuracy of serologic diagnosis of *H. pylori* infection in 695 school-aged children using ^{13}C-urea breath test as reference. They conclude that with an adjusted cut-off level for the enzyme-linked immunosorbent assay (ELISA), it was found to have an adequate performance in a pediatric population.

The rapid whole blood tests to detect antibodies against *H. pylori* have been used as a screening tool by primary care physicians and pediatricians, although they have not been recommended by published consensus conferences for both adults and children. Kindermann et al. [33] evaluated a rapid whole blood test (BM-Test). Almost half of the *H. pylori* infected and 10% of the non-infected children were misclassified by the BM-Test. False-negative results are not related to young age or ethnic groups. They conclude that poor performance makes the test unsuitable for epidemiological and clinical use in children.

Stool test

Data concerning the reliability of the noninvasive *H. pylori* stool antigen (HpSA) test in children were satisfactory [34]. Although HpSA is a convenient tool, especially for children, for the pretreatment diagnosis, however, its applicability in monitoring eradication therapy has been controversial as the assay can detect dead or partially degraded bacteria long after actual eradication, thus giving false positive results [35].

Konstantopoulos et al. [36] evaluated HpSA in a large cohort of children and compared it to invasive techniques and the ^{13}C-UBT. They found it to have a sensitivity of 88.9% and a specificity of 94% in symptomatic children, while for the ^{13}C-UBT it was 100% and 98.9%, respectively. However, in healthy toddlers, the HpSA performed as well as ^{13}C-UBT with excellent concordance. There was no age dependency of the stool results, and changing the cut-off did not improve the accuracy. Thus, HpSA seems suitable to monitor the success of anti-*H. pylori* therapy.

Salivary test

Data on the accuracy of salivary anti-*H. pylori* IgG antibodies are limited. Bode et al. [37] compared a salivary antibody test (ELISA) to ^{13}C-UBT. The sensitivity and specificity of the saliva test were 80.9% and 95.3%, respectively.

Treatment

No randomized controlled trials have been performed in children. Therefore, the 'steering committee' of the European Pediatric Task Force on *H. pylori* [38] announced the creation of the Paediatric European Register for treatment of *H. pylori* (PERTH) with the aim to collect data on efficacy of different regimens and to subsequently try to reach a consensus on treatment.

In a double-blind clinical study [39], a triple therapy, consisting of omeprazole, amoxicillin, and clarithromycin (OAC) for 1 week, was compared with a dual therapy of amoxicillin and clarithromycin (AC). Eradication of *H. pylori* was 75% and 9.4%, respectively. A lower eradication rate (64%) with AOC triple therapy of children in Brazil was found [40] in an open trial.

An effective triple therapy was found in an open, randomized study by Shcherbakov et al. [41]. The proprietary omeprazole (AstraZeneca) was more effective than the generic formulation (eradication 88.8% vs. 80.0%). An even higher eradication rate (94%) was found by Chan et al. [42] in a prospective study of a 1-week quadruple

therapy with omeprazole, clarithromycin, amoxicillin, and metronidazole.

The high rate of *H. pylori* resistance to antibiotic treatment regimens in children is a concern [43]. Thus, a European multicenter study on the resistance of *H. pylori* to antibiotics in children by the European Task Force on *H. pylori* is currently carried out.

In a European multicenter survey [44] of in vitro antimicrobial resistance in *H. pylori*, resistance to clarithromycin was higher in children (17.3%) and in teenagers (13.6%), in comparison to all other age groups (8.2%). A significantly higher rate of resistance to clarithromycin and metronidazole in *H. pylori* strains was found in natives of southern European countries.

High clarithromycin and metronidazole resistance have been found in different geographic areas [45,46]. PCR-RFLP had a high sensitivity (92%) and specificity (100%) for determination of clarithromycin resistance gene mutation, providing a rapid and accurate approach to detect clarithromycin-resistant strains within 24 hours [47].

Pretreatment antimicrobial resistance of *H. pylori* has been found to have a negative impact on treatment efficacy [48–50]. Whether the wide use of antibiotics for common infections influences the antibiotic resistance rate of *H. pylori* strains remains an unsolved question. Recently, Bontems et al. [51] studied the evolution of primary and secondary resistance in a large series of Belgian children during the last 12 years. Resistance to amoxicillin was not observed. The rate of resistance to imidazoles was 18% and remained constant throughout the period, whereas primary resistance to macrolides increased from an average of 6.0% before 1995 to 16.6% thereafter, which did not appear to correlate with macrolides prescription habits. Secondary resistance developed in 46%. In a similar 9 years study [52] on clarithromycin and metronidazole resistance of *H. pylori* in Spanish children, it was also increased in both. On the contrary, in a French study [45] the resistance rate to clarithromycin and metronidazole remained stable during the 5-year period from 1994 to 1999.

In strains isolated from patients with eradication failure, acquired resistance to the antibiotic used, especially clarithromycin, is frequently detected [51,53]. However, second line therapy for cases with eradication failure remains to be established. Fujimura et al. [54] studied the in vitro activity of rifampicin against *H. pylori* isolated from children and adults. They studied the susceptibility of 52 strains of *H. pylori* and all isolates were susceptible to rifampicin. None of the *H. pylori* isolates tested acquired rifampicin resistance during prolonged exposure to the drug in vitro. It is suggested that rifampicin has stable activity against *H. pylori*. However, further work would be required to establish its in vivo efficacy.

Conclusion

Epidemiological studies for *H. pylori* showed acquisition in early childhood. Most studies are in favor of intrafamilial transmission in young children and the *H. pylori* prevalence in the parental generation may be a crucial determinant for the child's risk of being infected. Infection is most common under crowded conditions and low socioeconomic status. Vertical transmission to the newborn does not seem possible.

Invasive tests remain the gold standard for diagnosis and among noninvasive tests, ^{13}C-UBT has the higher specificity and sensitivity in older children, but needs to be validated in children younger than 2 years. Selection of the proper commercial enzyme immunoassay increases the sensitivity of antibody detection. Rapid whole blood tests to detect antibodies against *H. pylori* have low sensitivity and should not be used.

Only one randomized controlled, double-blind trial was performed in children. Collaborative studies with a large number of children need to be undertaken. Primary and secondary resistance of *H. pylori* to antibiotics is increasing and is probably responsible for the treatment failure. Treatment should be adjusted according to the local susceptibility of *H. pylori* to antibiotics.

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Helicobacter pylori seroprevalence in patients with chronic bronchitis

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Background. A high *Helicobacter pylori* seroprevalence has been found in many extragastrointestinal disorders. Moreover, it has been reported that the risk of chronic bronchitis may be increased in *H. pylori*-infected patients. The aim of this study was to assess the *H. pylori* seroprevalence in patients with chronic bronchitis. ***Methods.*** We evaluated 144 patients with chronic bronchitis (81 men and 63 women, aged 53.2 ± 12.7 years) and 120 age and sex-matched control subjects. All enrolled subjects (bronchitic patients and controls) underwent an enzyme-linked immunosorbent assay (ELISA) IgG serologic test for *H. pylori* diagnosis. ***Results.*** A correlation between age and *H. pylori* IgG level was detected for both bronchitic patients ($r = 0.42$; $P = 0.004$) and controls ($r = 0.44$; $P = 0.004$). *H. pylori* seroposivity in the chronic bronchitis group was significantly higher than that in controls (83.3% vs 60%; $P = 0.007$). The mean serum concentration of IgG antibodies against *H. pylori* was also significantly higher in patients with chronic bronchitis than in the control subjects (38.7 ± 24.1 U/ml vs 25.9 ± 19.3 U/ml; $P = 0.02$). ***Conclusions.*** *Helicobacter pylori* infection may be associated with chronic bronchitis. Further studies should be undertaken to confirm our results and to clarify the potential underlying pathogenetic mechanisms.

Key words: chronic bronchitis, *Helicobacter pylori*, prevalence

Introduction

Helicobacter pylori infection of the gastric mucosa affects approximately 50% of the world's population.[1] It seems to be the main cause of chronic antral gastritis[2] and is strongly associated with peptic ulcer disease,[3] gastric cancer,[4] and gastric mucosa-associated lymphoid-tissue (MALT)—lymphoma.[5] A high *H. pylori* seroprevalence has also been found in many extragastrointestinal disorders, including coronary heart disease,[6] rosacea,[7] growth failure in childhood,[8] and active bronchiectasis.[9]

It is well known that the prevalence of chronic obstructive pulmonary disease in peptic ulcer patients is increased two-to-three fold compared with findings in ulcer-free controls.[10,11] The major factor underlying this association seems to be the impact of cigarette smoking on both diseases. However, a recent pilot study, in a small number of patients, showed that *H. pylori* infection, per se, might be related to an increased risk of developing chronic bronchitis.[12] An epidemiological study in Danish adults also suggested that chronic bronchitis might be more prevalent in *H. pylori* IgG-seropositive women than in uninfected ones.[13] However, insufficient information is available on the prevalence of *H. pylori* infection in chronic bronchitic patients.

In order to investigate the relation between *H. pylori* infection and chronic bronchitis, we assessed the *H. pylori* seroprevalence in a cohort of chronic bronchitics and control subjects.

Subjects and methods

The present study was conducted at the Ninth Department of Pulmonary Medicine, "Sotiria" Chest Diseases Hospital (Athens, Greece). The local ethics committee approved the study, and written informed consent was

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obtained from each participant. Following a predefined protocol, between March 1, 1998 and March 31, 2001, 192 consecutive patients with chronic bronchitis, diagnosed according to the American Thoracic Society Guidelines, were recruited from the outpatient clinics. Briefly, chronic bronchitis was diagnosed as "the presence of chronic productive cough for 3 months in each of 2 successive years, in a patient in whom other causes of chronic cough have been excluded."[14] Exclusion criteria were: (1) an exacerbation of chronic bronchitis in the preceding month (the exacerbation was defined as "increased dyspnea associated with change in the quality or quantity of sputum, which led the patient to seek medical attention[14]); (2) prior *Helicobacter* eradication therapy; (3) consumption of acid-suppressive drugs or antibiotics in the preceding 6 months; and (4) a history of vagotomy or operations on the upper gastrointestinal tract. A total of 48 patients were excluded. Therefore, 144 patients were eligible for analysis.

Controls were selected randomly from subjects who attended courses designed for public health education during the period of the study. Exclusion criteria for controls were: (1) a known history of chronic bronchitis and (2) a known history of gastrointestinal tract pathology. Finally, we selected 120 controls from among 195 healthy subjects and we matched them with the patients for sex, age (within 2 years), and socioeconomic status.

All subjects enrolled (bronchitic patients and controls) underwent an enzyme-linked immunosorbent assay (ELISA) IgG serologic test for *H. pylori* diagnosis (HEL-P test; Park, Athens, Greece), in accordance with the manufacturer's guidelines. Positive, borderline, and negative results were assigned when the concentration of IgG antibodies against *H. pylori* was greater than 20, between 12.5 and 20, and less than 12.5 U/ml, respectively. The specificity and sensitivity of the serology test, validated in our local population, were 95% and 85%, respectively.

Values for results are expressed as means ± 1 SD. The significance of differences between groups was assessed by unpaired Student's *t*-test for continuous variables and the χ^2 test for proportions. Correlation coefficients between variables were determined using conventional Pearson's correlation analysis. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program (SPSS, Los Angeles, IL, USA), and *P* values were two-tailed analyzed. *P* values of less than 0.05 were considered statistically significant.

Results

The demographic data of the patients and controls are shown in Table 1. There was no significant difference in age or sex distribution between the two groups. The majority of bronchitic patients were current cigarette smokers (94 patients; 65.2%) or ex-smokers (30 patients; 20.8%), and only 20 of the patients (14%) had never smoked. On the other hand, 72 of the 120 control subjects (60%) were never-smokers, 32 (26.6%) were current smokers, and 16 (13.4%) were previous smokers.

A correlation between age and *H. pylori* IgG level was detected for both bronchitic patients ($r = 0.42$; $P = 0.004$) and controls ($r = 0.44$, $P = 0.004$). Among the bronchitic patients, 120 (83.3%) were anti-*H. pylori* IgG-positive, 6 (4.1%) had borderline values, and 18 (12.5%) were seronegative. Of the control subjects, 72 (60%) had positive values, 3 (2.5%) were borderline, and 45 (37.5%) were seronegative.

Table 1 shows the analysis of the serologic parameters. *H. pylori* seroposivity in patients with chronic bronchitis was significantly higher than that in controls ($P = 0.007$). The mean serum concentration of IgG antibodies against *H. pylori* was also significantly higher in bronchitics than in control subjects ($P = 0.02$). Finally, *H. pylori* seroposivity did not differ significantly between current-smokers among the bronchitic patients and those patients with chronic bronchitis who had never smoked (86.1% vs 80%, respectively; $P = 0.9$)

Discussion

Data in the literature on the relationship between *H. pylori* infection and chronic bronchitis are poor. A previous epidemiological study, in a population of Danish women, suggested that *H. pylori* infection might

Table 1. Demographic data and *Helicobacter pylori* serologic parameters

Parameters	Controls ($n = 120$)	Bronchitics ($n = 144$)	*P* value
Age	50.8 ± 13.3	53.2 ± 12.7	0.81
Male sex (%)	57.5	56.2	0.31
H. pylori IgG level (U/ml)	25.9 ± 19.3	38.7 ± 24.1	0.02
H. pylori IgG seroposivity (%)	60	83.3	0.007

be associated with an increased prevalence of chronic bronchitis.[13] Recently, Gaselli et al.[12] carried out a prospective pilot study in a sample of 60 bronchitic patients and showed an increased *H. pylori* seroprevalence (81.6%) and an association of *H. pylori* infection with the risk of chronic bronchitis.

Our study is the first to have focused on the seroprevalence of *H. pylori* in a large population of patients with chronic bronchitis. According to our results, the *H. pylori* seroprevalence in bronchitic patients was significantly higher than that in the control subjects. The age-related pattern of infection, which, in our study, was detected for both bronchitics and controls, is common in developed countries, and is explained by the cohort effect.[15] The socioeconomic status, which is related to both *H. pylori* infection and the risk of chronic bronchitis, was similar in our two groups. Tobacco use could be another confounding factor. Cigarette smoking is the most important etiologic factor in chronic bronchitis, and seems to fully account for the association between peptic ulcer and chronic obstructive pulmonary disease observed in previous studies.[16,17] However, data on the relationship between *H. pylori* infection and smoking habits are controversial. The prevalence of *H. pylori* infection in smokers has been variously reported as low,[18] normal,[19] and high.[20] In the present study, we did not match bronchitics and control subjects for smoking habits. As the relationship between smoking and *H. pylori* infection has not yet been clarified, the possible impact of cigarette smoking on both chronic bronchitis and *H. pylori* infection should be regarded as a potential study limitation.

The present study has not focused on the potential pathogenetic mechanisms underlying the association between *H. pylori* infection and chronic bronchitis. This association might reflect either susceptibility induced by common factors or a kind of causal relationship between these diseases. As far as we know, there are no common factors implicated in the susceptibility to both chronic bronchitis and *H. pylori* infection. However, we cannot rule out this possibility, as the conditions predisposing to *H. pylori* infection have not been clarified yet. With regard to the potential etio-pathogenetic role of *H. pylori* infection in chronic bronchitis, the chronic activation of inflammatory mediators induced by *H. pylori* infection might lead to the development of a nonspecific inflammatory process such as chronic bronchitis. It is well known that *H. pylori* stimulates the release of a variety of proinflammatory cytokines, including interleukin-1 (IL-1), IL-8, and tumor necrosis factor-alpha, and the eradication of *H. pylori* leads to the normalization of serum cytokine levels.[21–23] These cytokines are also thought to be involved in the pathogenesis of chronic bronchitis.[24,25] Therefore, *H. pylori* infection might play a proinflammatory role and co-trigger chronic bronchitis with other more specific environmental, genetic, and unknown factors. Another potential pathogenetic mechanism could be the spilling or inhalation of *H. pylori* or its exotoxins into the respiratory tract, which also might lead to a chronic airway inflammation such as chronic bronchitis. However, as far as we know, neither the identification of *H. pylori* species in human bronchial tissue, nor the isolation of *H. pylori* from bronchoalveolar lavage (BAL) fluid has been achieved to date.[15] Studies estimating the relative risk of developing chronic bronchitis for *H. pylori*-infected patients, and the effect of *H. pylori* eradication on the natural history of chronic bronchitis are also needed to further investigate these hypotheses.

In conclusion, the present study suggests that patients with chronic bronchitis have an increased seroprevalence of *H. pylori* infection. Our results must be confirmed in a larger number of patients. Further studies should be undertaken to clarify the pathogenetic mechanisms underlying the possible association between these diseases.

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Helicobacter pylori infection in hospital workers over a 5-year period: correlation with demographic and clinical parameters

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Background. We aimed to determine whether any of various groups of medical and nonmedical staff in a large acute care hospital were at increased risk of acquiring *Helicobacter pylori* infection over a 5-year period, and we also aimed to identify risk factors or symptoms related to *H. pylori* positivity and seroconversion. ***Methods.*** A total number of 437 subjects, aged 36.8 ± 7.7 years (range, 23–60 years)—employees of our hospital—were tested by immunoassay for serum IgG antibodies against *H. pylori*. Subjects were assigned to four main groups: (I) nursing staff (n = 249; aged 34.7 ± 7 years); (II) administrative and technical staff (n = 127; aged 39.2 ± 8.1 years); (III) medical staff (n = 31; aged 42.4 ± 4.9 years); and (IV) paramedical staff (blood donor department) (n = 30; aged 37.6 ± 8.5 years). Differences in age and educational level between these four groups were statistically highly significant (P < 0.0001). Each subject completed a questionnaire containing several clinical and demographic parameters. The same cohort of individuals was tested 5 years later. ***Results.*** The overall seroprevalence of *H. pylori* infection was 45.5%, and in each group (I, II, III, and IV) being 48.6%, 44.1%, 41.9%, and 30% respectively. Logistic regression analysis revealed that the risk of infection by *H. pylori* was significantly higher in group I compared with group II (odds ratio [OR], 1.91; 95% confidence interval [CI], 1.04–3.52; P = 0.037). The *H. pylori* positivity increased with age: 40.6% for those aged 23–40 years and 57.5% for those aged 41–60 years (P = 0.001). The level of education was inversely associated with *H. pylori* infection (P = 0.001). During the 5-year observation, 59 of 238 (24.8%) subjects initially negative for *H. pylori* infection became positive, thus giving an annual seroconversion rate of 4.95%. Logistic regression analysis revealed that the seroconversion rate was significantly higher in group I compared with group II (28.1% vs 21.1%; OR, 2.34; 95% CI, 1.08–5.07; P = 0.03). The rate of seroconversion was higher in subjects aged 35–55 years compared with subjects aged 23–34 years (32% vs 17.5%; P = 0.009). Subjects who were positive for *H. pylori* infection in both examinations had a higher percentage of heartburn (P = 0.029), regurgitation (P = 0.023), and nausea (P = 0.037) compared with those who were negative in both examinations. Differences between those who were continuously negative for *H. pylori* infection and those who seroconverted during the observation period were not significant. ***Conclusions.*** In this longitudinal study of workers in a large acute care hospital in Greece it was found that nursing staff had a significantly higher risk of infection compared with administrative and technical staff. Age was significantly positively related both to *H. pylori* infection and to seroconversion. The level of education was strongly related to the prevalence, but not to the incidence of *H. pylori* infection. The presence of infection over the time was associated with a higher percentage of heartburn, regurgitation, and nausea compared with subjects who were continuously negative for *H. pylori* infection.

Key words: *Helicobacter pylori*, prevalence, incidence, epidemiology, high-risk groups, transmission, hospital workers, nursing staff, Greece

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Introduction

The mode and route of transmission of *Helicobacter pylori* is largely uncertain, although there is some evidence in favor of person-to-person transmission via the oro-oral, gastro-oral, or fecal-oral route.[1–10]

Staff involved in the care of patients are likely to be at a greater risk of acquiring the infection than the general population. Indeed, increased seroprevalence of *H. pylori* in nursing staff and gastroenterologists has been reported in some studies,[11–16] although an equally large number of studies did not show any significant correlation between occupation and *H. pylori* infection.[17–23]

All studies performed so far simply reflect the situation at the time of examination, which is a comparison of the prevalence of infection with *H. pylori* among medical and nursing staff and of the general population at a given time. So far, there has been no study comparing the rate of infection by *H. pylori* in the same cohort of hospital workers over a certain period of time. Moreover, there is no clearly recognized syndrome associated with chronic *H. pylori* infection.[24,25] Whether the seroconversion is associated with symptoms of any kind is also largely unknown.

This study was specifically designed to investigate the prevalence of *H. pylori* seropositivity in nursing, medical, paramedical, and administrative staff in a large acute care hospital at two different time periods and in the same cohort of individuals. Another aim of the study was to record a large number of demographic and clinical parameters, as well as personal habits, in order to see whether there was any correlation between existing or new symptoms and established or newly acquired *H. pylori* infection. Questions arising during the design of this study were as follows: Is the nursing and/or medical staff at increased risk of acquiring infection by *H. pylori* compared with administrative staff? Is there any correlation between certain demographic and clinical parameters and personal habits with *H. pylori* infection over time? What is the rate of seroconversion in hospital workers, a group that is likely to be at increased risk of acquiring infective diseases? In order to answer these questions we examined a large number of hospital workers during two different time periods (first trimester of the years 1994 and 1999). The study was approved by the Ethics Committee of our hospital.

Subjects and methods

Subjects

The initial study population consisted of 493 persons, employees of our hospital. They were assigned to four main groups: (I) Nursing staff (persons working in the wards or special units), (II) administrative and technical staff (persons working in the offices or other departments of the hospital without any contact with the patients), (III) medical staff (doctors working in the medical departments), and (IV) paramedical staff (persons working in the blood donor department).

In the 1999 study, we intended to include all 493 persons examined in 1994. However, 56 of them were lost to follow-up for reasons mainly related to their movement to other parts of the country, thus leaving a total number of 437 persons for analysis. There were 141 (32.3%) men and 296 (67.7%) women, of mean age 36.8 ± 7.7 years (range, 23–60 years). The nursing staff consisted of 249 individuals, the administrative and technical staff consisted of 127 individuals, the medical staff consisted of 31 individuals, and the paramedical staff consisted of 30 individuals. The nursing staff was further divided into three subgroups: nurses working in endoscopy and other special units (subgroup A; $n = 66$), wards (subgroup B; $n = 114$), and outpatient clinics (subgroup C; $n = 69$). During the 5-year period of observation, almost all nurses (97%) were working in the same ward or department.

Questionnaire

A self-report questionnaire was complete by all subjects at the same time as their blood specimen was taken. The questionnaire included a large number of items, such as age, sex, number of brothers and sisters, educational level, smoking habits, coffee and alcohol consumption, medical history with special emphasis on diseases of the digestive system, surgery, and various chronic disorders. We also recorded many other details and symptoms in relation to the digestive system, including number of bowel movements, consistency of stools, heartburn, regurgitation, nausea, bloating, peptic ulcer, gastritis, and dietetic habits (Mediterranean diet). The Mediterranean diet is a well-known diet adopted by people living in the Mediterranean area, and includes seven main constituents; namely, bread, olive oil, fruits, milk, pulses, wine, and vegetables.[26] Diagnoses such as duodenal or gastric ulcer and gastritis were reported by the study participants and did not necessarily reflect endoscopic or radiologic diagnoses. All participants gave their informed consent to the study.

Measurement of serum antibodies against H. pylori

Blood specimens were obtained from each participant by the usual venipuncture. After centrifugation, serum was stored at −60°C until the time of analysis. Each serum specimen was coded and the same number was given to the questionnaire so that identification of the participants was not possible by the laboratory staff or other persons, except for the person responsible for the collection of the data.

Serum antibodies against *H. pylori* were measured using an enzyme immunoassay kit (Pyloragen Higher

Biomedica, Hycor Biomedical GmbH, Kassel, Germany). The test was assigned as positive if the value of IgG antibodies in the serum was greater than 40 U/ml. Although the sensitivity and specificity of the immunoassay were greater than 95% and 96%, respectively, according to the manufacturer, the two parameters were again validated in our laboratory and were found to be greater than 96%. The test for the detection of *H. pylori* infection in both periods of time was the same. Tests were performed by the same laboratory staff and under the same conditions.

Statistical analyses

A three-step analysis was undertaken. In order to select variables for a multivariate analysis and illustrate clinically important comparisons that might be lost in a regression equation, we first computed univariate comparisons, including χ^2 tests for nominal data (e.g., marital status and educational level) and *t*-tests (*P* values were two-tailed) for continuous measures such as age. We compared these groups with respect to *H. pylori* infection at the first stage of the analysis (1994). To summarize the findings of the study, a logistic regression analysis was next calculated, using the variables with significant univariate comparisons as independent variables and the results of *H. pylori* tests as the dependent variable. This was done in order to determine the study variables that best differentiated the staff members with and without *H. pylori* infection. Use of this analysis allowed for a simultaneous assessment of discriminating variables while controlling for collinearity variables for any possible confounding (e.g., age, marital status), and to avoid the limitations of multiple comparisons. A stepwise procedure was used to arrive at the final model. The third step was to compare the independent variables with *H. pylori* infection 5 years later (1999) in order to investigate the role of these variables in *H. pylori* infection, with the same procedures. Logistic regression analysis was applied in order to determine risk factors for *H. pylori* seroconversion. The same statistical model was applied, in order to assess the possible association between symptoms and *H. pylori* infection.

Results

Initial examination (1994)

Prevalence of H. pylori *infection*

The mean age of the four groups tested (nursing, administrative, medical, and paramedical staff) was 34.7 ± 7, 39.2 ± 8.1, 42.4 ± 4.9, and 37.6 ± 8.5 years, respectively. Differences concerning the mean age of the four groups were statistically highly significant ($P < 0.0001$). Differences concerning the educational level were also highly significant ($P < 0.0001$).

The overall seropositivity of *H. pylori* was 45.5% (199 of 437 subjects). The rate of infection in the nursing, administrative, medical, and paramedical staff was 48.6%, 44.1%, 41.9%, and 30%, respectively ($\chi^2 = 4.13$; degrees of freedom [df] = 3; $P = 0.25$). The mean age of the infected subjects was significantly higher than that of those not infected (38.1 ± 7.9 vs 34 ± 6.7 years, respectively; $P < 0.0001$). The rate of infection increased significantly with age: 40.6% for those aged 23–40 and 57.5% for those aged 41–60 years ($P = 0.001$). No significant differences in the prevalence of *H. pylori* infection by sex were noted (65 out of 141; 46.1%, in males vs 134 out of 296; 45.3%, in females).

Risk factors for acquiring H. pylori *infection*

Six of the 118 risk variables examined; namely, age, educational level ($P = 0.001$), number of siblings (2.94 ± 2 vs 2.41 ± 1.6; $P = 0.003$), history of hypertension (10.2% vs 2.3%; $P = 0.001$), duodenal ulcer (13% vs 4.5%; $P = 0.002$), and adoption of the so-called Mediterranean diet (16.3% vs 6.9%; $P = 0.007$) were found to be statistically significantly related to *H. pylori* infection on univariate analyses (χ^2 and *t*-tests). Subjects who were positive for *H. pylori* infection were older, were less educated, and had more siblings compared with subjects who were negative for *H. pylori* infection. Other parametric and nonparametric variables, such as sex; marriage; smoking; number of cigarettes per day; instant coffee and Greek coffee consumption; surgical operations; history of gastric ulcer; constipation; surgical operations, including appendicectomy,tonsilectomy, cholecystectomy, and gastrectomy; chronic disorders, such as obstructive lung disease, myocardial infarction, diabetes mellitus, and cerebral stroke; and positive history of hyperlipidemia, were not significantly related to *H. pylori* infection. Logistic regression analysis confirmed the strong association of age and educational level with *H. pylori* positivity. As is obvious in Table 1, nurses had an increased risk of infection by *H. pylori* compared with administrative and technical staff (OR, 1.91; 95% CI, 1.04–3.52; $P = 0.037$).

Further analysis showed that the risk of infection by *H. pylori* was significantly higher in subgroup A (nurses working in special units) compared with subgroup C (nurses working in outpatients clinics) (adjusted, for age and education, OR, 2.13; 95% CI, 1.02–4.4; $P = 0.04$) but not between subgroups B (nurses working in wards) and C (OR, 1.75; 95% CI, 0.92–3.3; $P = 0.09$).

Symptoms

As far as the relationship between the presence of digestive symptoms and *H. pylori* infection was con-

Table 1. Results of logistic regression analysis of the study variables and their association with *Helicobacter pylori* seroprevalence

	Odds ratio	95% Confidence interval (CI)	*P*
Age	1.04	1.003–1.08	0.03
Education			
Higher[a]	—		
Elementary	3.53	1.53–8.10	0.01
Moderate	1.41	0.78–2.54	NS
Profession			
Administrative staff[a]	—		
Nurses	1.91	1.04–3.52	0.037
Doctors	1.29	0.46–3.63	NS
Paramedical staff	1.03	0.31–3.44	NS
Number of siblings	1.05	0.91–1.21	NS
History of hypertension			
No[a]	—		
Yes	2.40	0.70–8.15	NS
Mediterranean diet			
No[a]	—		
Yes	2.14	0.95–4.78	NS

NS, Not significant
[a] Referent group

Table 2. Seroconversion of *H. pylori* infection within each professional group over the 5-year period

Group	Number of new cases	
	Over the 5-year period	Per year of observation
Nursing staff (n = 128)	36 (28.1%)	5.6%
Administrative staff (n = 71)	15 (21.1%)	4.2%
Doctors (n = 18)	3 (16.6%)	3.3%
Paramedical staff (n = 21)	5 (23.8%)	4.7%
Total (n = 238)	59 (24.8%)	4.95%

Note: Between professional group comparisons, χ^2 = 1.92; degrees of freedom (df) = 3; NS

cerned, statistically significant differences were noted only for symptoms of heartburn (29.1% vs 18.4%; P = 0.013) and bloating (33% vs 23.5%; P = 0.036). Adjusted ORs for sex and age were 1.65; 95% CI, 1.02–2.69; $P < 0.04$, for heartburn and 1.55; 95% CI, 0.98–2.45; P = 0.056, for bloating.

Re-examination (1999)

Prevalence of H. pylori *infection*

The overall seropositivity for *H. pylori* infection in the same cohort of individuals as that observed in 1994 when seen in the 1999 examination was 57.7% (252/437) (group I, 61% (152/249); group II, 55.1% (70/127); group III, 51.6% (16/31); and group IV, 46.7% (14/30). Although differences between the four groups tested were not statistically significant on univariate analysis (χ^2 = 3.45; df = 3; P = 0.32), logistic regression analysis after adjustment for age and educational level revealed significant differences between nursing and administrative staff (OR, 1.94; 95% CI, 1.19–3.16; P = 0.007). Further analysis showed that differences between nursing subgroups A and C were significant (66% vs 56.5%, adjusted OR, 2.25; 95% CI, 1.05–4.82; P = 0.03), but not between subgroups B and C (60.5% vs 56.5%; OR, 1.41; 95% CI, 0.73–3.32; P = 0.29). Among the remaining variables only age and educational level were significantly related to *H. pylori* seropositivity.

Seroconversion rate

Positive. During the 5-year observation, 59 of 238 (24.8%) subjects initially negative for *H. pylori* infection became positive, thus giving an annual seroconversion rate of 4.95% (Table 2).

Negative. A total number of six subjects initially positive for *H. pylori* infection became negative. However, three of them became negative as a result of eradication treatment. Analysis of the medical history of the other three individuals revealed that they had received

antibiotic treatment (once, once, and twice, respectively) for upper respiratory infection. Thus, the annual negative seroconversion rate was 0.15% (3 of 196 initially positive for *H. pylori* infection).

Risk factors predisposing to positive H. pylori *seroconversion*

We also studied a number of possible risk factors for acquiring *H. pylori* infection during the observation period. Univariate analyses revealed that the mean age of seroconvertors was statistically significantly higher as compared with the mean age of nonseroconvertors (37.1 ± 6 vs 34.8 ± 7 years; $P = 0.02$). In more detail, the percentage of seroconversion was significantly higher in subjects aged 35–55 compared with subjects aged 23–34 (32% [38/118] vs 17.5% [21/120]; $P = 0.009$). The percentage of subjects who had undergone appendicectomy was higher in those who seroconverted compared with those who had not (42.4% vs 25.8%; $P = 0.03$). Logistic regression analysis with the dependent variable being the seroconversion and independent variables age, profession, educational level, sex, and history of appendicectomy, revealed that age and occupation were the only significant risk factors predisposing to seroconversion (Table 3). As can be seen in Table 3, nursing staff had a significantly increased risk of seroconversion compared with administrative staff (OR, 2.34; 95% CI, 1.08–5.07; $P = 0.03$). Further analysis showed that differences in the rate of seroconversion between the three subgroups of nurses (A, B, and C) were not statistically significant (34% vs 28% vs 25%).

Symptoms

As far as the presence of symptoms was concerned, subjects were divided into three groups: (1) those who remained *H. pylori*-negative, (2) those who seroconverted, and (3) those who remained *H. pylori*-positive during the 5 years' observation. Differences between these three groups are shown in Table 4. As indicated in Table 4, subjects in group 3 had a significantly higher proportion of heartburn, regurgitation, and nausea compared with group 1 (Adjusted ORs for sex and age were: 1.73; 95% CI, 1.04–2.88; $P = 0.035$, for heartburn; 2.25, 95% CI 1.27–4.0; $P = 0.005$, for regurgitation; and 2.31; 95% CI, 1.21–4.44; $P = 0.01$, for nausea respectively). Differences between groups 1 and 2 were not statistically significant.

Discussion

To the best of our knowledge, this is the first study performed so far that has been specifically designed to investigate the prevalence of *H. pylori* infection over a 5-year period in a large cohort of healthy individuals working at an acute care hospital. Another aim of the study was to examine the relationship (if any) of the

Table 3. Risk factors predisposing to seroconversion from *H. pylori*-negative to *H. pylori*-positive in 5 years among healthcare workers at the General Hospital of Nicea, Greece (according to multivariate analysis)

	Odds ratio	95% CI	*P*
Age	1.07	1.02–1.13	0.008
Profession			
Administrative staff[a]	—		
Nurses	2.34	1.08–5.07	0.03
Doctors	1.01	0.19–5.11	NS
Paramedical staff	1.23	0.37–4.16	NS
Education			
Higher[a]	—		
Elementary	1.23	0.40–3.69	NS
Moderate	1.56	0.72–3.39	NS
Sex			
Male[a]	—		
Female	1.24	0.58–2.66	NS
Appendicectomy			
No[a]	—		
Yes	1.78	0.91–3.47	NS

NS, Not significant
[a] Referent group

Table 4. Gastrointestinal symptoms and *H. pylori* infection in subjects who remained *H. pylori*-negative, those who seroconverted, and those who remained *H. pylori*-positive

Symptoms	*H. pylori*-negative subjects ($n = 179$)	Newly infected subjects ($n = 59$)	*H. pylori*-positive subjects ($n = 193$)	*P* value
Heartburn	21.3%	22.9%	33.9%	0.029
Regurgitation	17%	18.8%	29.4%	0.023
Nausea	11.1%	12.5%	21.2%	0.037
Vomiting	3.3%	4.2%	5.9%	NS
Bloating	35.9%	22.9%	41.8%	NS
Borborygmus	18.3%	14.6%	20%	NS

Note, The six negative seroconvertors were excluded from the analysis
NS, Not significant (χ^2 test between-group comparisons)

previously or newly acquired *H. pylori* infection with symptoms or gastrointestinal disorders and other risk factors. The cohort of individuals examined corresponded to almost 50% of the total number of people working in the hospital. However, the number of doctors tested was quite low, due to their obstinately negative desire to participate in the study.

Rate of infection by H. pylori

Previous studies concerning the rate of *H. pylori* infection in hospital workers produced conflicting results. So, increased rates of *H. pylori* infection have been observed in a number of studies concerning nurses, nurses working at endoscopy laboratories, endoscopists, gastroenterologists, and medical staff.[11–16] Nevertheless, an equally large number of studies did not find higher infection rates in medical staff and persons working at endoscopy units, compared with normal controls.[17 23]

Our results showed that an increase in the risk of *H. pylori* infection among nurses certainly exists. This conclusion can be derived from the higher rate of infection observed in nursing staff compared with administrative and technical staff on multivariate analysis, although univariate analysis did not show any significant differences. However, this can be explained by the highly statistically significant differences in the age and educational level of the various groups of hospital workers. It is of interest that the rate of infection was found to be significantly different between different groups of nurses. So, nurses working in special units such as intensive care, hemodialysis, and endoscopy had higher rates of infection compared with nurses working in the outpatients' clinic. This is in accordance with some studies claiming that nurses working in endoscopy units,[12,27] as well as in other special units, such as hemodialysis,[28] have an increased risk of developing *H. pylori* infection.

Factors related to H. pylori *infection*

In our study we were able to identify some risk factors that were positively or negatively related to *H. pylori* seroconversion. Age, number of siblings, hypertension, and adoption of the so-called Mediterranean diet, were all found to be positively related to *H. pylori* infection. Education was found to be "protective" against infection by *H. pylori* on univariate analysis. However, after adjustment for other confounding factors, only age and educational level remained significantly related to *H. pylori* infection.

Age is a well-known epidemiological feature strongly related to *H. pylori* infection.[29] In the study of Gasbarrini et al.,[28] *H. pylori* infection among healthcare workers was associated with two factors, namely, age and level of father's education. Our study showed a statistically significantly positive correlation of *H. pylori* infection with age and a negative one with educational level. We also noted a positive correlation between *H. pylori* infection and number of siblings in all groups of individuals, a finding compatible with current views.[1–3] It seems that *H. pylori* infection can be transmitted most readily among siblings who are close in age, and this occurs most frequently from older to younger ones.[30] According to recent reports, the risk of infection increases with the number of years lived with an infected partner.[31] However, in our study, marriage was not significantly related to *H. pylori* acquisition. In accordance with most of the relevant studies, no significant differences in the rate of infection by *H. pylori* between men and women were noted,[28] although opposite views certainly exist.[30] Various types of diet have been investigated during the past few years in relation to *H. pylori* infection.[32–39] Some of them have been found to be protective,[35,36,38] while others were not.[34,39] On multivariate analysis, we showed that the adoption of the so-called Mediterranean diet did not have any significant influence on the rate of infection by *H. pylori*.

Seroconversion rate

The overall incidence of *H. pylori* positivity in the hospital staff between the years 1994 and 1999 was 24.8%, thus giving an annual rate of infection of 4.95%. The rate of infection among the nursing group was significantly higher than that in the administrative staff. So, nursing staff must be considered as a high-risk group for *H. pylori* seroconversion. As far as the medical and paramedical staff are concerned, although the rate of seroconversion was lower compared with the nursing group, no conclusive results could be derived, as the number of subjects examined was quite small.

We were not able to identify in the available literature studies concerning the rate of seroconversion in hospital workers. However, a number of previous studies have examined the development of infection in the general population using two measurements separated by some years. The period between the two testings ranged from 2 to 20 years. The incidence of new infections by *H. pylori* in developed countries has been estimated to range between 0.5% and 1.1% yearly,[40] although in developing countries the rate of infection is higher, at 3%–10%.[41] In a recently published study from a developing country, the early re-infection rate following successful eradication of *H. pylori* in duodenal ulcer patients was similar (8%) to that reported in countries with a low prevalence of infection.[42] Other studies found that the annual rate of infection in endoscopy staff was 2.6%,[16] while it was 0.3% in the general population.[43] In Canada, the crude annual seroconversion rate was 1%.[44] It is of interest that a high seroconversion rate (7.3%) was noted in the United States military staff who served

in the Persian Gulf war in 1990 and 1991.[45] In Japan, the annual seroconversion rate for children and adults has been estimated to be 1.8% and 1.5%, respectively.[46] On the contrary, in Denmark, changes in *H. pylori* infection with time are rare.[47] Becker et al.[48] found an annual seroconversion rate of 1.9% in a cohort of 312 North American missionaries who were serving in developing countries between 1967 and 1984. A quite high seroconvertsion rate (2.3%) in trainee nurses was described in a recently published study from Germany.[49]

So far, there are no available data concerning the seroconversion rate in the general Greek population. The rate of seroconversion found in our study, although quite high, must not be considered an unexpected one, and it fits well with that described in previous studies concerning blood donors and the healthy Greek population. The seroprevalence of *H. pylori* infection in healthy Greek adults has been estimated to be between 60% and 70% in blood donors and healthy individuals aged between 20 and 50 years.[50,51] In a recently published study concerning Greek military personnel (a group at high risk for acquiring *H. pylori* infection) a seroconversion rate of 14.8% among 142 young male navy recruits was found 8 months after their induction.[52]

In our study, risk factors found on univariate analysis to be statistically significantly related to positive seroconversion were age and previous appendicectomy. However, on logistic regression analysis, age and profession were the factors related to *H. pylori* seroconversion. The role of age was further supported by the significantly increased rate of seroconvertion observed in subjects aged 35–55 years compared with those aged 23–34 years. This role of age is further supported by the higher rate of *H. pylori* positivity found in subjects over 40 years of age. We are not able to give a satisfactory answer to the question of why subjects aged 35–55 have a higher rate of seroconversion compared with subjects aged 23–34. However, Gasbarrini et al.[28] reported that age older than 35 years was the only independent predictor of the likelihood of *H. pylori* positivity. Results similar to ours concerning the role of age and seroprevalence of *H. pylori* were also described by Rudi et al.[19] This finding probably reflects the assumption that some aspects of living conditions have improved considerably in the younger part of this population compared with the older one. Moreover, is possible that younger nurses probably adopted safer practices in patient care. We think that further studies are needed in order to confirm our findings and to offer a more logical explanation.

Symptoms

In our study, heartburn, regurgitation, and nausea were the only symptoms positively related to *H. pylori* infection in those individuals who were positive for *H. pylori* infection on both examinations. Other studies have claimed that *H. pylori* infection is accompanied by heartburn and nausea,[47] although it is possible that some of these individuals may, in fact, suffer from functional dyspepsia. Luzza et al.[53] found that only dyspepsia, peptic ulcer, occupation, crowding, and number of siblings were associated with *H. pylori* infection. Rudi et al.[19] emphasized that, with the exception of peptic ulcer, no other risk factor for *H. pylori* infection is important. It is worth noting that, in their study, the majority of subjects who were found to be positive for *H. pylori* infection remained asymptomatic. Parsonnet et al.,[54] in a cohort study of epidemiologists, found that persistent negatives to *H. pylori* described symptoms similar to these described by persistent positives, although subjects who seroconverted in the interval between the two serum samples were more likely to have experienced upper gastrointestinal symptoms in the intervening years. In our study, no significant difference in the presence of symptoms between subjects negative for *H. pylori* infection and those who acquired the *H. pylori* infection within the 5-year period were found. We suggest that longterm *H. pylori* infection is probably more important for the development of symptoms in the upper gastrointestinal tract than the newly acquired infection.

In summary, our results showed that, among the various groups of hospital workers, nurses appeared to have a higher risk of infection by *H. pylori* compared with administrative and technical staff, thus supporting the assumption of person-to-person transmission. Our results confirmed previous observations claiming that age and low educational level were positively related to *H. pylori* seroprevalence. Age and profession (nursing staff) were found to be the only significant factors predisposing to seroconversion during the 5-year period of our study. The presence of infection by *H. pylori* over the time is associated with a higher rate of heartburn, regurgitation, and nausea, compared with findings in subjects who are continuously negative for *H. pylori* infection. Whether our findings can be universally applied deserves further investigation.

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JOURNAL OF CLINICAL GASTROENTEROLOGY

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Helicobacter pylori Seroprevalence in Patients With Pulmonary Tuberculosis
[Letters to the Editor]

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To the Editor:

A recent epidemiologic study suggests that a history of tuberculosis (TB) may be associated with increased prevalence of Helicobacter pylori infection. 1 Although increased risk of TB for persons with a history of peptic ulcer disease has also been reported, insufficient information is available on the prevalence of H. pylori infection in TB patients. 2 A previous study showed no difference in H. pylori seroprevalence between patients on anti-TB chemotherapy and control subjects. 3 However, anti-TB regimens including rifampicin and streptomycin may eradicate H. pylori infection and subsequently decrease serum concentrations of H. pylori immunoglobulin G (IgG) antibodies. 4 To investigate the relation between H. pylori infection and TB, we assessed the H. pylori seroprevalence in newly diagnosed TB patients before the initiation of anti-TB treatment.

Following a predefined protocol, between January 1, 1998, and January 31, 2001, we studied all consecutive patients with newly diagnosed pulmonary TB. Our control group included healthy subjects, well-matched for age, sex, and socioeconomic status. All subjects enrolled (TB patients and controls) underwent an enzyme-linked immunosorbent assay IgG serologic test for H. pylori diagnosis (HEL-P test; Park Co, Athens, Greece), in accordance with the manufacturer's guidelines. A positive, borderline, and negative result was assigned when the concentration of IgG antibodies against H. pylori was more than 20 U/mL, between 12.5 and 20 U/mL, and less than 12.5 U/mL, respectively. The specificity and sensitivity of the serologic test validated in our local population were 95% and 85%, respectively.

A total of 80 TB patients and 70 control subjects were recruited into this study. The demographic data of both patients and controls are shown in Table 1. There was no statistical difference in age or gender between the two groups.

Parameters	Controls (n = 70)	TB patients (n = 80)	p value
Age (y)	56.8 ± 15.9	55.2 ± 14.8	0.78
Male gender (%)	54.3	57.5	0.42
H. pylori IgG level (U/mL)	26.1 ± 21.2	39.0 ± 25.2	0.02
H. pylori IgG seropositivity (%)	61.4	87.5	0.007

TABLE 1. Demographic data and H. pylori serologic parameters

Among the TB patients, 70 (87.5%) were anti-H. pylori IgG-positive, 2 (2.5%) had borderline values, and 8 (10%) were seronegative. Of the control subjects, 43 (61.4%) had positive

values, 2 (2.9%) were borderline, and 25 (35.7%) were serative. A correlation between age and H. pylori IgG level was detected for both TB patients (r = 0.42, p – 0.004) and controls (r = 0.44, p = 0.004). Table 1 shows analytically the serologic parameters. The H. pylori seropositivity in the TB group was significantly higher than that of controls (p = 0.02). The mean serum concentration of IgG antibodies against H. pylori was also significantly higher in TB patients (p = 0.007).

Our study is the first focused on the seroprevalence of H. pylori in TB patients, before the initiation of anti-TB treatment. According to our results, the H. pylori seroprevalence in patients with pulmonary TB is significantly higher than that of the control subjects. The socioeconomic status is similar between the two groups. A possible common route of transmission could be another confounding factor. However, neither H. pylori nor Mycobacterium tuberculosis has been cultured from traditional environmental reservoirs (such as water, insects, pets, or farm animals). 5,6 Moreover, the airborne transmission of M. tuberculosis occurs even without physical contact, whereas H. pylori spreads via close physical contact. Thus, the observed association between H. pylori infection and pulmonary TB seems to be real and irrespective of transmission-associated confounding factors.

The current study has not focused on the potential pathogenetic mechanisms underlying the association between Helicobacter infection and development of pulmonary TB. This association might reflect susceptibility to both infections induced by common host genetic factors. It has been suggested that HLA-DQ serotype may contribute to enhanced mycobacterial survival and replication. 7 This serotype is also associated with increased susceptibility to H. pylori infection. 8 On the other hand, the role of chronic H. pylori infection as a predisposing factor for development of pulmonary TB is unknown. An increased risk of TB for persons who had undergone partial gastrectomy or vagotomy for peptic ulcer disease has been reported. 9 Some authors suggest that the risk of TB may be increased in patients with a history of peptic ulcer disease, irrespective of surgery. 2 With regard to the pathogenetic role of H. pylori infection in peptic ulcer disease, the above studies led to a hypothesis that H. pylori infection, per se, may be related to the risk of pulmonary TB.

However, the common genetic predisposition to both bacteria and the consideration of H. pylori infection as a predisposing factor for development of pulmonary TB are only hypotheses. We cannot exclude the possibility that other factors, linked to the host or bacterial strains, may be responsible for the observed association between the two infections.

In conclusion, the current study suggests that patients with pulmonary TB have an increased seroprevalence of H. pylori infection. Our results must be confirmed in a larger number of patients. Further studies should be undertaken to clarify the pathogenetic mechanisms underlying the possible association between H. pylori infection and pulmonary TB.

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JOURNAL OF CLINICAL GASTROENTEROLOGY

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The Incidence of Helicobacter pylori Infection in Greek Female Patients With Autoimmune Hypothyroidism: Is There a Relationship?

[Letters to the Editor]

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Outline

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- Fig. 1

To the Editor:

Thyroid autoantigens have shown some degree of cross-reactivity with bacterial ones, 1 whereas a relationship between Helicobacter pylori infection and the development of autoimmune atrophic thyroiditis or vice versa has been reported. 2,3

We studied 98 Greek women (age range, 21–80 years; mean age, 46.38 ± 13.88 years) with autoimmune thyroiditis, documented by the presence of clinical and biochemical hypothyroidism and positive titer of antithyroid autoantibodies (antithyroglobulin normal value, < 100 U/mL; anti-TPO normal value, < 30 U/mL). All patients were receiving L-T4 treatment and had thyroid-stimulating hormone (TSH) levels within the normal range. The measurements of TSH, anti-TPO, and antithyroglobulin were performed by immunoradiometric assay techniques (Diasorin s.r.l., Italy). All patients were evaluated for the presence of H. pylori infection by determination of serum anti-H. pylori immunoglobulin G (IgG) levels using enzyme-linked immunosorbent assay. Anti-H. pylori IgG values greater than 10 U/mL were considered indicative of H. pylori infection.

In the 20 patients with gastrointestinal symptoms (of the 63 positive for H. pylori infection), eradication therapy was administered (amoxicillin 1 g B.I.D., clarithromycin 500 mg B.I.D., lansoprazole 30 mg B.I.D., all for 10 days). Six months later, antithyroid autoantibody and anti-H. pylori IgG levels were re-evaluated. The eradication therapy proved successful in all patients (reduction of the anti-H. pylori IgG at levels below the cutoff point of 10 U/mL). Statistical analysis was performed by [chi]2 test; statistical significance was accepted as $p < 0.05$. Values are expressed as mean and standard deviation (± SD).

Sixty-three of 98 patients with autoimmune thyroiditis were positive for H. pylori infection (64.28%). Comparing these results with the published data for the seroprevalence of H. pylori IgG antibodies in a representative sample of healthy Greek women (57%), 4 no statistical significance was documented, either in all of the patients ($p = 0.297$) or in specific age groups (21–40 years, $p = 0.422$; 41–60 years, $p = 0.491$; > 60 years, $p = 0.509$) (Fig. 1). In patients who received eradication therapy for H. pylori infection, the observed slight fluctuation in anti-TPO and antithyroglobulin plasma titer 6 months later, although present, was not statistically significant, and it could be the result of other contributing factors (chronic therapy for thyroid dysfunction, etc.). 5

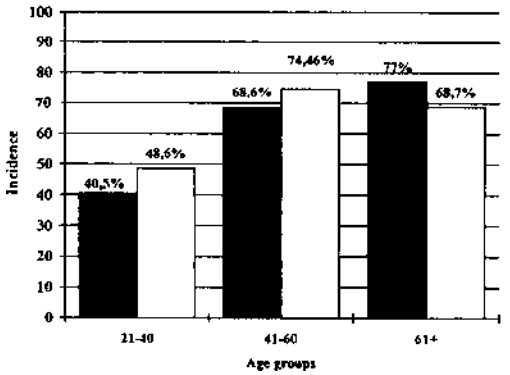

FIG. 1. Incidence of positive Helicobacter pylori immunoglobulin G antibodies in different age groups of a representative population of healthy Greeks ([black small square]) and of Greek women with autoimmune hypothyroidism ([white square]) (n = 98). (The prevalence of H. pylori infection is equally distributed between men

and women in the general population.) For the 21 to 40 years of age group, the p value was 0.422; 41 to 60 years, p = 0.491; and 611, p = 0.509. Some data from Archimandritis et al. 4

Thyroid autoantigens have shown some degree of cross-reactivity with bacterial antigens. 1 An association of both autoimmune thyroid disease and H. pylori infection with mucosa-associated lymphocyte T lymphomas has been reported, as well as gastric mucosa-associated lymphoid tissue in autoimmune thyroid disease. 6 Some other investigators recently found that monoclonal antibodies to an H. pylori strain with CagA positivity reacted with follicular cells of the thyroid gland and that an H. pylori organism possessing the CagA pathogenicity island carried a gene encoding for an endogenous peroxidase. They concluded that CagA-positive H. pylori infection increases the risk of autoimmune thyroid disease development. 3

Our results, however, do not support the hypothesis of an etiologic link between autoimmune thyroiditis and H. pylori infection; we found that there is no greater incidence of H. pylori infection in patients with autoimmune hypothyroidism compared with the general population of the same sex, age, and nationality. Moreover, in the patients with autoimmune hypothyroidism who received eradication therapy for H. pylori infection, there was no statistically significant change in antithyroid antibody titers. The nonsignificant fluctuations noted are probably related to the natural course of the chronic autoimmune disease. 5

To our knowledge, this is the first clinical study evaluating the prevalence of H. pylori infection in a large number of patients with Hashimoto thyroiditis, with regard to its prevalence in the general, homogenous population of their country. In the one clinical study published so far, a markedly increased prevalence of H. pylori infection has been reported in patients with autoimmune thyroid disease (Hashimoto and Graves'), but the control group consisted of patients with multinodular goiter and Addison's disease. 2

However, if an etiologic association between H. pylori infection and Hashimoto thyroiditis exists, perhaps it could be revealed if patients at an early stage of the disease were studied. Initiation of the autoimmune process, which could be triggered by H. pylori infection, precedes—often for a long time the establishment of hypothyroidism and is indicated only by the presence of positive antithyroid antibodies. Another possibility could be that only specific subtypes of H. pylori and special factors of the host, such as human leukocyte antigen, must coexist to produce local or general disorders.

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Volume 35(5) November 2002 pp 706-707

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The Role of Helicobacter pylori in Nonulcer Dyspepsia

[Letters to the Editor]

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Outline

We read with interest the Editorial by Splawski (1) entitled "Helicobacter pylori and Nonulcer Dyspepsia: Is There a Relation?" However, we disagree with the author's approach to support a relation between H.pylori and nonulcer dyspepsia by showing that eradication of H.pylori results in resolution of symptoms, although the existing data do not support that H.pylori infection is a significant cause of abdominal pain or dyspepsia (2,3), neither do they establish that H.pylori is a primary pathogen of underlying gastritis (4–6).

Recently, we have shown that 93% (4) to 94% (5) of studied children with recurrent abdominal pain or dyspepsia had an underlying chronic inflammatory lesion (e.g., esophagitis, gastritis or duodenitis, separately or combined) which could explain their complaints (5). H.pylori infection was found in 7% (4) to 15% (5) of the studied patients.

The data of our studies (4,5) show an increased incidence of upper gastroduodenal mucosal inflammation among the children studied, considering that an absolutely normal gastric mucosa is found only in the first decade of life (7). These findings simply demonstrate a strong association between upper gastroduodenal mucosal inflammation and abdominal pain or dyspepsia.

To see whether healing of underlying chronic inflammatory lesion results in resolution of symptoms, 14 of the studied children with recurrent abdominal pain completed a single blind placebo-controlled clinical trial (5). All these children underwent endoscopic, esophageal gastric, and duodenal biopsy before and after treatment with ranitidine (n = 9) or placebo (n = 5) for 2 months. The results of this trial, although the number of patients was small, showed that ranitidine was more effective than placebo and that healing of underlying chronic inflammatory lesion was found to be associated with resolution of dyspeptic symptoms or abdominal pain. In addition, in another treatment study of 64 of the 71 studied children with recurrent abdominal pain or dyspepsia, 12 received ranitidine for two months and 52 received for three months (4). All of them were responsive within one week of starting therapy. Of the 64 patients, eight relapsed between 1 and 8 months (mean 3.5) after discontinuation of ranitidine and 11 between 9 and 27 months (mean 20.4) leaving 37 children who were found to be symptom free and without medication for over 17 to 40 months (mean 26.3) Contact has been lost with eight families. These data clearly demonstrate a causal association of upper gastrointestinal chronic mucosal inflammation and recurrent abdominal pain or dyspepsia.

The data of our studies (4,5), consistent with those of others (2,3), do not support an association between H.pylori infection and recurrent abdominal pain or dyspepsia; neither do they support an association between H.pylori and upper gastrointestinal mucosal inflammation seen in the children studied. However, our histologic findings demonstrate that unknown factors, other than H.pylori, are most frequently involved in the genesis of inflammatory lesion seen in the children studied. These findings and the characteristic pattern of distribution of H.pylori colonization on the gastric mucosa of studied children are consistent with those of our study in children with migraine (6). They provide strong evidence that H.pylori organisms may not be primary pathogens of gastritis and raise the possibility that H.pylori organisms may colonize gastric tissue as a result of inflammation rather than as a cause of it. Further evidence to support this contention is provided by the data seen in 1 of the 14 aforementioned patients with recurrent abdominal pain who completed a single blind placebo-controlled clinical trial (5). This patient, who was infected by H.pylori during the trial, showed, at the end of treatment with placebo, nodular antral gastritis (via endoscopy), and an increase of pre-existing mild antral gastritis to moderate one, with colonization of

H.pylori and mucosal lymphoid follicles (via histology). The pre-existing mild corporal gastritis and mild duodenitis remained as they were before colonization of H.pylori occurs (Table 5)(5). All these findings do not support that H.pylori is a primary cause of gastritis. However, they demonstrate that H.pylori is an aggravating cause of gastritis and explain the reason why the data of recent studies in children (8) and adults (9–12) show that eradication of H.pylori infection is not followed by the healing of gastritis although a significant improvement of the underlying gastritis is noted.

In conclusion, our findings do not support an association between H.pylori and abdominal pain or dyspepsia; neither do they establish a causal link between H.pylori and underlying gastritis or gastroduodenitis. However, our data demonstrate a causal association between chronic inflammatory lesion and abdominal pain or dyspepsia. They also provide evidence that H.pylori infection, as an aggravating cause of gastritis, should be eradicated and the underlying inflammatory lesion, representing most probably the pre-existing gastritis, should be further treated for resolution of symptoms, using a potent anti-ulcer drug. Long-term, well-controlled multicenter trials will be required so that we will be able to find out the proper treatment of nonulcer dyspepsia with or without infection.

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LETTERS

No increased prevalence of *Helicobacter pylori* in patients with alopecia areata

To the Editor: Thirty patients with alopecia areata (AA) and 30 age- and sex-matched healthy volunteers were examined for the presence of IgG antibodies to *Helicobacter pylori* with the use of a commercially available in-office test (ImmunoComb II, Orgenics), with sensitivity of 92% and specificity of 93.1%. Both groups were comparable in terms of age (31.5 vs 32 years) and sex (50% women in both).

According to seropositivity for IgG antibodies, there was no statistical difference ($P = .3015$) between the two groups. Gastrointestinal symptoms, concominant diseases, and habits in the group of patients and the prevalence of *H pylori* antibodies are listed in Table I.

The hypothesis of the autoimmune nature of AA is supported not only by the coexistence of the disease with other autoimmune diseases, but also with the presence of autoantibodies against thyroid constituents, gastric parietal cells, and smooth muscle cells.[1,2] The autoimmune nature of the disease is also supported by the identification of antibodies that are directed against normal anagen scalp hair follicles in the serum of patients with AA.[3] In addition, the serum levels of cytokines in the localized forms (interleukins [IL] 1α and 4) and the extensive forms (interferon gamma and IL-2) of the disease are significantly elevated.[4] De Luis et al[5,6] have published two articles showing that the seroprevalence of *H pylori* is significantly higher in patients with insulin-dependent diabetes mellitus and autoimmune atrophic thyroiditis. They express the hypothesis that *H pylori* antigens might be involved in the development of these two autoimmune diseases or that autoimmune function in these diseases may increase the likelihood of an *H pylori* infection. On the basis of these studies and considering the fact that AA is a disease of unknown origin, we tried to determine whether there might be an association of *H pylori* infection with AA.

We found no significant difference in the seroprevalence of *H pylori* infection between patients with AA and healthy controls.

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Table I. Prevalence of IgG *Helicobacter pylori* antibodies and concomitant diseases, habits, and gastrointestinal symptoms

	No. of patients (n = 30)	*H pylori* IgG positive
Vitiligo	2	1
Hashimoto's thyroiditis	1	1
Atopic dermatitis	1	0
Hyperthyroidism	2	1
Diabetes mellitus	1	0
Indigestion or heartburn	5	4
Anxiety	13	7
Smoking	10	6
Family history of peptic ulcer	2	0
Use of antacids	5	4

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False-negative rapid urease test in acute upper GI bleeding: Is human plasma alone responsible?

To the Editor:

We read with interest the article by Houghton et al.[1] regarding the bacteriocidal effect of human plasma against *Helicobacter pylori*. The aim of their study was to explain the clinical observation that the sensitivity of the rapid urease test (RUT) for the detection of *H pylori* is decreased in patients with acute upper GI bleeding (UGB). We were among the first to report the lower sensitivity of biopsy-based methods (RUT, histology) as compared with serology for detection of *H pylori* infection based on a study of a cohort of 55 consecutive patients with acute UGB.[2] We further found that histology was significantly more sensitive than the RUT in the determination of *H pylori* status in patients with acute UGB, irrespective of age or the presence of blood in the stomach at endoscopy.[3] Moreover, the sensitivity of the RUT was low in patients who were bleeding irrespective of the use of nonsteroidal anti-inflammatory drugs (NSAIDs). However, in patients with acute UGB who were not using NSAIDs, the sensitivity of the RUT remained low, but histopathologic evaluation of appropriately stained (modified Giemsa) biopsy specimens was positive for *H pylori* significantly more often than in bleeding patients who were taking NSAID (77% vs. 52%, $p = 0.047$; 95% CI [0.013, 0.468]). This led us to speculate that NSAIDs may have a direct effect on *H pylori* viability, or that the decreased sensitivity of histology in patients taking NSAIDS is due to sampling error, which may occur more often when biopsy specimens are taken endoscopically from a stomach with NSAID-induced gastropathy. Thus, we believe that other factors, in addition to the bacteriocidal effect of human plasma proposed by Houghton et al.,[1] contribute to the observed low sensitivity of the RUT during acute UGB.

When endoscopy is performed for acute UGB, we propose that at least 2 additional biopsy specimens be obtained from the antrum and kept in formalin. If the RUT is negative, these specimens should be processed for histopathologic evaluation. Alternatively, in this clinical setting, locally validated noninvasive tests, such as serology, could be used for the evaluation of *H pylori* status with the objective of early initiation of eradication therapy in infected patients, and the consequent prevention of recurrent ulcer bleeding with its ramifications for less hospitalization and treatment.

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