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Trends in the prevalence of atrophic gastritis and *Helicobacter pylori* infection over a 10-year period in Japan: The ROAD study 2005-2015

IZUMI INOUE[1], NORIKO YOSHIMURA[2], TOSHIKO IIDAKA[2], CHIAKI HORII[3], SHIGEYUKI MURAKI[2], HIROYUKI OKA[4], HIROSHI KAWAGUCHI[5], TORU AKUNE[6], TAKAO MAEKITA[7], KANAE MURE[8], KOZO NAKAMURA[9], SAKAE TANAKA[3] and MASAO ICHINOSE[7]

[1]Health Service Center, Tokyo University of Marine Science and Technology, Tokyo 108-8477; [2]Department of Prevention Medicine for Locomotive Organ Disorders, 22nd Century Medical and Research Center; [3]Department of Orthopedic Surgery, Sensory and Motor System Medicine, Graduate School of Medicine; [4]Department of Medical Research and Management for Musculoskeletal Pain, 22nd Century Medical and Research Center, The University of Tokyo, Tokyo 113-8655; [5]Department of Orthopedic Surgery, Tokyo Neurological Center, Tokyo 105-0001; [6]Department of Orthopedic Surgery, National Rehabilitation Center for Persons with Disabilities, Saitama 359-0042; Departments of [7]Gastroenterology and [8]Public Health, School of Medicine, Wakayama Medical University, Wakayama 641-0012; [9]Department of Orthopedic Surgery, Towa Hospital, Tokyo 120-0003, Japan

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Abstract. Few large population-based studies have examined the prevalence of atrophic gastritis (AG) and *Helicobacter pylori* infection in Japan. The purpose of the present study was to estimate the prevalence of AG and *H. pylori* infection by age, in addition to investigating their change rates from 2005 to 2016 in Japan using data from a large population-based cohort. A total of 3,596 participants [1,690 in the baseline survey (2005-2006) and 1,906 at the fourth survey (2015-2016)] aged 18 to 97 years were included in the cohort. The prevalence of AG and *H. pylori* infection were examined at baseline and in the fourth survey based on serological tests for the *H. pylori* antibody titer and pepsinogen levels. The prevalence of AG and *H. pylori* infection were 40.1% (men, 44.1%; women, 38.0%) and 52.2% (men, 54.8%; women, 50.8%), respectively, at baseline. AG seropositivity rates showed a significant decrease from 40.1 to 25.8% in 10 years. *H. pylori* seropositivity rates decreased significantly from 52.2 to 35.5% in 10 years. Stratified for age, the prevalence of AG showed an increasing trend with age, whereas the prevalence of *H. pylori* infection increased with aging, except for in the elderly group, showing an inverted U-shaped association. In this population-based, cross-sectional study with a 10-year interval survey, the prevalence of AG and *H. pylori* infection decreased significantly. This change may influence the prevalence of *H. pylori*-related diseases, including extra-gastric disorders associated with *H. pylori*-induced systemic subclinical inflammation and hypochlorhydria, such as colorectal neoplasia and arteriosclerosis.

Correspondence to: Professor Izumi Inoue, Health Service Center, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan
E-mail: iinoue0@kaiyodai.ac.jp

Abbreviations: AG, atrophic gastritis; BMI, body mass index; CI, confidence interval; EIA, enzyme immunoassay; *HP, Helicobacter pylori*; IgG, immunoglobulin G; OR, odds ratio; PG, pepsinogen; RIA, radioimmunoassay; SD, standard deviation

Key words: cohort, pepsinogen, atrophic gastritis, *Helicobacter pylori*, prevalence

Introduction

Helicobacter pylori is known to be a major cause of gastric cancer development and may contribute to extra-gastric organ disease. Previous large-scale prospective cohort studies have shown that *H. pylori* is a definite risk factor for gastric cancer, and it is widely recognized that atrophic gastritis (AG) associated with progression of *H. pylori*-related gastritis significantly increases the risk of cancer (1-4). In recent years, it has also been shown that decreased gastric acid secretion evaluated by pepsinogen (PG) is associated with colorectal carcinogenesis (5,6) and arteriosclerosis-related diseases such as diabetes mellitus (7). The prevalence of *H. pylori* has been reported to vary by race and country (8, 9) and the incidence has decreased markedly in the developed world over recent decades (10). The prevalence of *H. pylori* in Japan is lower than in developing countries and higher than in other developed countries (11). There were several studies reporting the prevalence of *H. pylori* in rural and urban areas of Japan (12-16). Clarifying epidemiological indicators, such as the prevalence and secular

trend, of the disease is the first step in disease prevention. *H. pylori* infections and AG are often characterized by few symptoms, chronic progression, and a long course. Therefore, estimation of these epidemiological indicators requires population-based cohort screening and long-term observation. However, only a few studies have investigated the prevalence of *H. pylori* in a large population-based subjects in Japan (16). As for the prevalence of AG, there are very few reports on the natural history of AG in a cohort of local residents and no reports on long-term prognosis. The definitive diagnosis of *H. pylori*-related gastritis and resulting gastric atrophy is based on histopathology of the gastric mucosa. However, it is difficult to accurately diagnose the severity and progression of *H. pylori*-related AG by histopathology of several endoscopically collected specimens since AG develops multifocally, in addition histopathology-based diagnosis of AG involves subjective assessment without a gold standard (17). There is general agreement that the serum *H. pylori* antibody titer is believed to be related to the activity of inflammation in *H. pylori*-related gastritis (18,19) and serum PG levels show dynamics that correlate with histopathological changes and exocrine function in the gastric mucosa corresponded to the extent of gastric atrophy (20-23). In other words, serum PG are regarded as markers reflecting the progression of chronic AG. Therefore, the present study used these serum markers, which are more objective parameters, free of discomfort, easy to accept and relatively inexpensive for mass population. Measuring serum PG is the only viable method for estimating the prevalence of functional AG in the large general population (24). Song *et al* reported a statistically significant decreasing trend in the prevalence of functional atrophic corpus gastritis defined by serum PG I measurements in the age group of 55 to 64 years from 1990 through 2009 based on a population-based study in Sweden (25). In Japan, the prevalence of AG is expected to increase due to further aging of the population, but no report has examined the prevalence of AG in a large-scale, population-based samples.

The Research on Osteoarthritis/Osteoporosis Against Disability (ROAD) study started in 2005 for the purpose of prevention of musculoskeletal diseases and identification of risk factors. This is based on an ongoing prospective survey in the general population being conducted in Wakayama Prefecture, located in the southwestern part of the main island of Japan. The first baseline survey was conducted in 2005-2006. Participants in the ROAD study were informed to undergo follow-up surveys at 3, 7, and 10 years after enrollment, when the baseline examinations were repeated. The fourth survey was the 10-year follow-up and was conducted in 2015-2016. Participants in this study were similar to the general Japanese population in terms of physique, alcohol consumption and other lifestyle habits (26). Wakayama Prefecture is one of the high-risk areas for gastric and colorectal cancer mortality in Japan; in 2005, gastric and colorectal cancer mortality ranked within the top five among 47 prefectures in Japan, respectively (27). Thus, the prevalence of AG and *H. pylori* infection using this large-scale population-based results could be generalizable to the Japanese population in a high-risk area for gastric and colorectal cancers.

The purpose of this study was to estimate and compare the latest prevalence and secular trends of functional AG and *H. pylori* infection determined by serological results for *H. pylori* antibody and PG test with a 10-year interval using the baseline and fourth survey data of the ROAD study.

Patients and methods

Study subjects. The present study involved the ROAD study cohorts established in 2005. The ROAD study is a national, prospective study of musculoskeletal diseases in Japan, consisting of a population-based cohorts. Profiles of this longitudinal cohorts were detailed in previous reports (28,29,30). Briefly, a baseline database consists of the clinical and genetic information of 1,690 residents surveyed between 2005 and 2006. The subjects were 1,690 participants reported in the ROAD study, recruited with reference to the lists of resident registrations in two communities: 864 participants from a mountainous area in Hidakagawa, Wakayama Prefecture and 826 participants from a coastal area in Taiji, Wakayama Prefecture. Since the fourth survey is both 10-year follow-up to the baseline study and a new baseline database for the subsequent 10-year period (the parent ROAD of this study is an ongoing cohort study), in the fourth survey, new participants recruited from the resident registration records of the two communities using the same method as the baseline survey were included. Therefore, the fourth survey included 979 new cohort participants in addition to 927 cohort followers. In this population-based cross-sectional study with a 10-year interval survey, a total of 1,690 subjects in the baseline survey and 1,906 in the fourth survey who underwent blood examination were initially included. Fig. 1 shows schematic flow of subject's recruitment and survey of this study. Participants were essentially asymptomatic for gastrointestinal symptoms requiring prompt medical care and could be regarded as representative of healthy middle-aged and older persons in the general population. They answered an interviewer-administered questionnaire consisting of lifestyle information such as drinking habits, family history and medical history. Height and weight were measured, from which the body mass index (BMI) [weight (kg)/height $(m)^2$] was calculated. According to the questionnaire at the time of the fourth survey, 131/1906 (6.9%) of the subjects had a history of *H. pylori* eradication therapy and we excluded these *H. pylori* eradicated subjects from the analysis. At the time of the health check, blood samples were obtained from the participants. Serum samples were isolated from blood taken as routine laboratory tests for the general health examinations and stored below -20°C until measurement of serum *H. pylori* immunoglobulin (Ig) G antibody titers and serum PG levels. In this study, blood samples at the baseline survey and the fourth survey were used. However, two subjects in the baseline survey and two subjects in the fourth survey with an insufficient amount of stored serum were excluded from PG testing and serological testing for *H. pylori* infections. Finally, a total of 1,557 subjects in the baseline survey and 1,773 subjects in the fourth survey were used for analysis in this study. Written informed consent was provided by all participants prior to inclusion. This study was performed in accordance with the Declaration of Helsinki and was approved by the institutional ethics committees of the University of Tokyo (approval nos. 1264 and 1326), Wakayama Medical University (approval no. 373) and Tokyo University of Marine Science and Technology (approval no. 187).

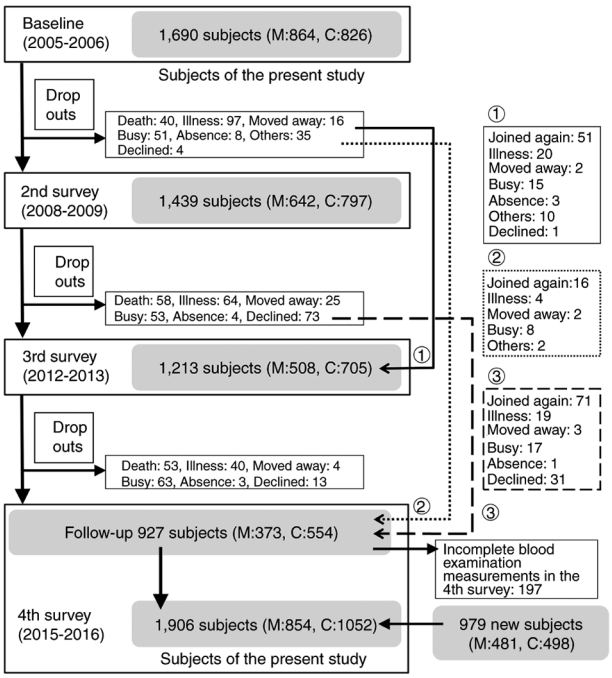

Figure 1. Schematic flow of subject's recruitment and survey. M, mountainous area; C, coastal area.

Serological analysis. Serum *H. pylori* antibody titers were measured using an enzyme immunoassay (EIA kit; SRL, Tokyo, Japan) (31). Antibody titers above 10 U/ml were classified as positive for *H. pylori* infection. Serum PG I and PG II levels were measured using a modification (RIA-Beads Kit; Dainabot, Tokyo, Japan) of radioimmunoassay (32). The gold standard for detecting gastric atrophy is still histopathology (biopsy) of the gastric mucosa, which was not selected in this study. However, there is general consensus that serologic testing with pepsinogen may help identify patients with AG (33). A recent meta-analysis of 31 studies involving a total of 2,265 AG patients showed that the summary sensitivity and specificity of AG screening with serum pepsinogen were 0.69 (95% CI: 0.55-0.80) and 0.88 (95% CI: 0.77-0.94) respectively, indicating that serum pepsinogen may be useful for noninvasive diagnosis of AG (34). Participants with AG were determined based on PG test-positive criteria of PG I ≤70 ng/ml and PG I/II ratio ≤3.0 (23). These criteria offer sensitivity of 70.5% and specificity of 97% for the diagnosis of AG (23). The criterion for the PG test used in the present study is the one most widely used for the detection of AG in Japan and considered a reliable non-invasive screening tool for AG.

Statistical analysis. Data for continuous variables are presented as means ± standard deviation (SD), and the differences were tested for significance using unpaired t-tests (Student's t-test and Welch's t-test) for comparisons of two groups. Differences in proportions were compared using the χ^2 test. The odds ratios (ORs) were estimated by a logistic regression model. ORs and 95% confidence intervals (CIs) were calculated using logistic regression analysis. P<0.05 was considered to indicate a statistically significant difference. Data analyses were performed using SPSS version 27.0 software (SPSS, Chicago, IL) and STATA (STATA Corp., College Station, TX).

Results

Background of study population. Table I shows the characteristics of this study subjects used for analysis. There were 1,557 participants at baseline and 1,773 in the fourth survey, for a total of 3,330 participants. There were no significant differences by gender between the two groups. The mean age was 65.70 (SD 12.17) years at baseline and 64.59 (SD 12.91) years in the fourth survey, with significant difference between the two. Current smokers accounted for 12.6% at baseline and 9.0% in the fourth survey, and inhabitants of coastal regions accounted for 47.5 and 54.5%, respectively. At baseline, smoking tended to be more frequent, and the percentage of subjects living in a coastal community was lower (P<0.01). The percentage of non-drinkers and the mean BMI tended to be higher in participants at baseline (P<0.1). Therefore, smoking habits, alcohol use, BMI, and community were also included in the model to control for confounding effects in the subsequent analyses. The serum PG I or II level was significantly higher at baseline than in the fourth survey, whereas the PG I/II ratio was significantly lower at baseline.

Prevalence of AG and H. pylori infection with a 10-year interval survey. Table II shows a comparison of the prevalence of AG and *H. pylori* infection at baseline and in the fourth survey. The prevalence of subjects with AG diagnosed by the PG test was significantly lower in the fourth survey (25.8%) compared to the baseline survey (40.1%), with a crude OR of 0.52 (95% CI: 0.45-0.60), and the significance of the difference did not change after adjustment. The percentage of *H. pylori* infection was 35.5% in the fourth survey and 52.2% at baseline, with a crude OR of 0.51 (95% CI: 0.44-0.58), and the significance of the difference did not change after adjustment (Table II). No significant gender differences were observed in the prevalence of AG and *H. pylori* infection (Table III). Of a total of 1690 patients in the baseline survey (864 mountain, 826 coastal), 927 (373 mountain, 554 coastal) also participated in the fourth survey, therefore the follow-up rate for the same patients was 54.9% (43.2% in mountainous areas, 67.1% in coastal areas). The prevalence of AG and *H. pylori* infection in these follow-up participants was 33.5% (mountain 39.1%, coastal 29.8%) and 52.5% (mountain 56.0%, coastal 50.1%), respectively, at baseline and in the fourth survey, they were 27.9% (34.9% in mountain, 23.3% in coastal) and 36.4% (40.5% in mountain, 33.6% in coastal), respectively. From the above results, the follow-up cases (total, mountainous area, coastal area) that participated in both the baseline and fourth survey showed almost the same tendency as the results of overall participants. Subjects in mountainous areas had a significantly higher mean age than subjects in coastal areas, and the prevalence of AG and *H. pylori* infection in mountainous areas tended to be higher than in coastal areas (Table SI).

Fig. 2A shows the age-specific prevalence of AG across the two surveys spanning 10 years. The prevalence of AG by the PG test was significantly higher with age at baseline than in the fourth survey (P<0.01), and there was no significant difference between men and women (data not shown). Stratified by age, there were 105 subjects (baseline survey: fourth survey=45:60) <40 years old, 317 (134:183) in their 40s, 594 (265:329) in their 50s, 979 (429:550) in their 60s, 953 (525:428) in their 70s, and

Table I. Comparison of background characteristics of the participants in the baseline survey (2005-2006) with those in the fourth survey (2015-2016).

Characteristic	Baseline survey	Fourth survey	P-value[a]
Total number of subjects	1,557	1,773	
Age, years[b]	65.70 (12.17)	64.59 (12.91)	<0.05
BMI, kg/m^{2}[b]	23.03 (3.42)	22.83 (3.53)	0.09
H. pylori antibody, U/ml[b]	19.78 (23.04)	14.91 (19.66)	<0.01
PG I, ng/ml[b]	58.47 (39.96)	55.14 (51.99)	<0.05
PG II, ng/ml[b]	20.87 (13.88)	15.30 (13.49)	<0.01
PG I/II[b]	3.19 (1.78)	4.03 (1.86)	<0.01
Sex, Men/Women[c]	531/1,026 (34.1/65.9)	572/1,201 (32.2/67.8)	0.27
Community, Mountain/Coastal[c]	817/740 (52.5/47.5)	807/966 (45.5/54.5)	<0.01
Current smoking habit, -/+[c]	1,326/191 (87.4/12.6)	1,611/160 (91.0/9.0)	<0.01
Current alcohol use, -/+[c]	946/603 (61.1/38.9)	1,013/758 (57.2/42.8)	<0.05
H. pylori infection, -/+[c]	745/812 (47.8/52.2)	1,143/630 (64.5/35.5)	<0.01
Atrophic gastritis, -/+[c]	933/624 (59.9/40.1)	1,315/458 (74.2/25.8)	<0.01

[a]Two-sided P-values for the difference between the baseline and 4th survey were determined using χ^2 test or t-test. [b]Data are presented as mean ± SD; [c]data are presented as count (%). *H, Helicobacter*; PG, pepsinogen; BMI, body mass index; SD, standard deviation.

Table II. Comparison of the prevalence of *Helicobacter pylori* infection or atrophic gastritis between the baseline survey (2005-2006) and the fourth survey (2015-2016).

Variable	Baseline survey, n (%)	Fourth survey, n (%)	OR[a] (95%CI)	OR[b] (95%CI)
H. pylori infection				
(-)	745 (47.8)	1143 (64.5)	1 (Ref)	1 (Ref)
(+)	812 (52.2)	630 (35.5)	0.51 (0.44-0.58)	0.51 (0.44-0.59)
Atrophic gastritis				
(-)	933 (59.9)	1315 (74.3)	1 (Ref)	1 (Ref)
(+)	624 (40.1)	458 (25.8)	0.52 (0.45-0.60)	0.53 (0.45-0.61)

ORs of the 4th survey were determined using logistic regression analysis. [a]Unadjusted. [b]Adjusted for age, sex, community, current smoker and alcohol use, and BMI by logistic regression analysis. *H, Helicobacter*; BMI, body mass index; OR, odds ratio; CI, confidence interval.

382 (159:223) ≥80 years old. The prevalence of AG-positive increased with age in both groups at baseline and in the fourth survey. The prevalence rates of AG-positive by age groups of <40, 40-49, 50-59, 60-69, 70-79, and ≥80 years were 11.1, 20.9, 29.4, 42.2, 46.5, and 55.3%, respectively, at baseline and 3.3, 11.5, 17.0, 25.6, 34.3, and 40.8%, respectively, in the fourth survey. AG-positive rates were significantly lower in the 10-year follow-up group than in the baseline group, in each age grade among subjects aged 40 years or older, with crude ORs according to age groups of <40, 40-49, 50-59, 60-69, 70-79, and ≥80 years of 0.28, 0.49, 0.49, 0.47, 0.60, and 0.56, respectively.

Fig. 2B shows the age-specific prevalence of *H. pylori* infection in two surveys at 10-year intervals. The prevalence of *H. pylori* infection indicated an increasing trend with age, except for the elderly group over 60 years old, in both the baseline and fourth surveys. The prevalence rates of *H. pylori* infection according to age groups of <40, 40-49, 50-59, 60-69, 70-79, and ≥80 years were 15.6, 32.1, 46.4, 61.3, 57.0, and 48.4%, respectively, at baseline and 8.3, 19.1, 31.6, 39.5, 41.4, and 41.3%, respectively, in the fourth survey. *H. pylori* seropositive rates were significantly lower in the fourth survey than at baseline, in each age group among subjects aged 40 years or older except for the oldest group, with a crude OR according to age groups of <40, 40-49, 50-59, 60-69, 70-79, and ≥80 years of 0.49, 0.50, 0.53, 0.41, 0.53, and 0.75, respectively. Next, to clarify whether the prevalence of *H. pylori* infection in the elderly over 60 years old did not increase with age as shown in Fig. 2B because of the effect of natural eradication, subjects aged 60 years and older were divided into age groups 60-69, 70-79, and 80 years and older. Then each age group were classified into subgroups based on the presence or absence of AG and *H. pylori* infection status, and for each survey, we then compared the prevalence of subgroups within each age group (Table IV). In the baseline survey, the prevalence of *HP* (+) AG (+) or *HP* (-) AG (-) did

Table III. Comparison of the prevalence of *Helicobacter pylori* infection or atrophic gastritis between the baseline survey (2005-2006) and the fourth survey (2015-2016).

A, Men

Variable	Baseline survey, n (%)	Fourth survey, n (%)	OR[a] (95%CI)	OR[b] (95%CI)
H. pylori infection				
(-)	240 (45.2)	372 (65.0)	1 (Ref)	1 (Ref)
(+)	291 (54.8)	200 (35.0)	0.44 (0.35-0.57)	0.45 (0.35-0.58)
Atrophic gastritis				
(-)	297 (55.9)	427 (74.7)	1 (Ref)	1 (Ref)
(+)	234 (44.1)	145 (25.3)	0.43 (0.33-0.56)	0.46 (0.35-0.60)

B, Women

Variable	Baseline survey, n (%)	Fourth survey, n (%)	OR[a] (95%CI)	OR[b] (95%CI)
H. pylori infection				
(-)	505 (49.2)	771 (64.2)	1 (Ref)	1 (Ref)
(+)	521 (50.8)	430 (35.8)	0.54 (0.46-0.64)	0.53 (0.45-0.64)
Atrophic gastritis				
(-)	636 (62.0)	888 (73.9)	1 (Ref)	1 (Ref)
(+)	390 (38.0)	313 (26.1)	0.58 (0.48-0.69)	0.57 (0.47-0.69)

ORs of the 4th survey were determined using logistic regression analysis. [a]Unadjusted. [b]Adjusted for age, sex, community, current smoker and alcohol use, and BMI by logistic regression analysis. *H, Helicobacter*; BMI, body mass index; OR, odds ratio; CI, confidence interval.

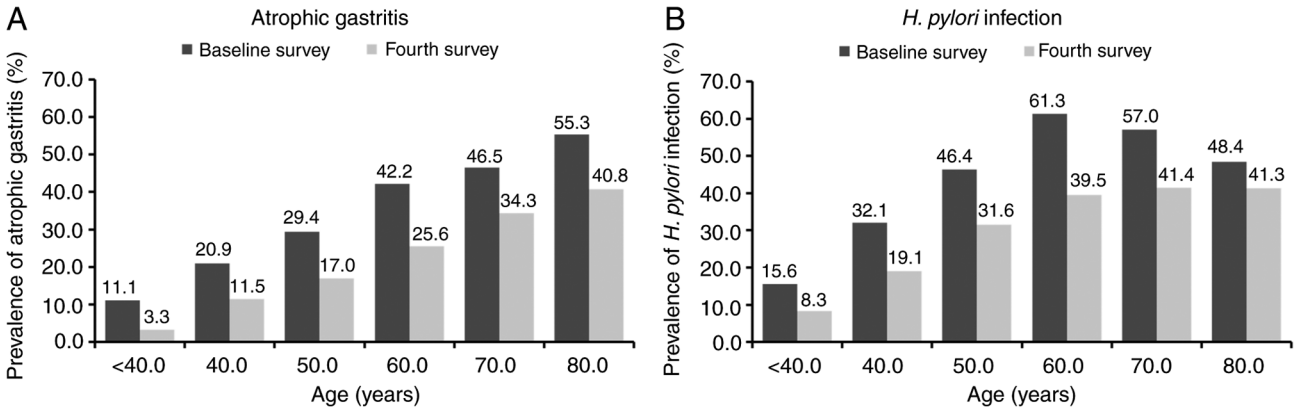

Figure 2. Comparison of the age-stratified prevalence of (A) atrophic gastritis or (B) *H. pylori* infection between the baseline survey (2005-2006) and the fourth survey (2015-2016).

not differ significantly among age groups 60-69, 70-79, and 80 years and older. On the other hand, the prevalence of *HP* (-) AG (+) showed a remarkably increasing trend with age, whereas the prevalence of *HP* (+) AG (-) showed a decreasing trend with age. From the above results, the prevalence of *HP* (+) decreased, and the prevalence of *HP* (-) AG (+) increased significantly with age in the elderly aged 60 years and over. So, prevalence was thought to exhibit an inverted U-shaped relationship (as shown in Fig. 2B). At the fourth survey, the prevalence of *HP* (-) AG (-) or *HP* (+) AG (-) decreased with age, whereas *HP* (+) AG (+) or *HP* (-) AG (+) showed an increasing trend with age. As a result, as shown in Fig. 2B, it was thought that the prevalence of *H. pylori* infection was not significantly different between the age groups of the elderly. In both the baseline and the fourth surveys, the prevalence of *HP* (-) AG (+) tended to increase with age in the elderly and the *HP* (-) AG (+) subgroup proportions were significantly higher in the 70-79 and ≥80 age groups compared with the 60-69 age group. [Baseline survey: Crude OR 2.04 (1.26-3.29) for age group 70-79 years vs age group 60-69 years, crude OR 2.93 (1.63-5.29) for age group ≥80 years] [Fourth survey: Crude OR 2.51 (1.48-4.28) for age group 70-79 years vs. age group 60-69 years, crude OR 3.50 (1.94-6.31) for age group ≥80 years].

Table IV. Comparison of the prevalence of subgroups classified by *Helicobacter pylori* infection and atrophic gastritis between age groups 60-69 years and 70-79 years or ≥80 years in each survey.

A, Baseline survey

		Age, years			Age, years	
HP	AG	60-69, n (%)	70-79, n (%)	≥80, n (%)	70-79, OR (95%CI)	≥80, OR (95% CI)
(-)	(-)	135 (31.5)	154 (29.3)	49 (30.8)	1 (Ref)	1 (Ref)
(+)	(-)	113 (26.3)	127 (24.2)	22 (13.8)	0.99 (0.70-1.39)	0.54 (0.31-0.94)
(+)	(+)	150 (35.0)	172 (32.8)	55 (34.6)	1.01 (0.73-1.38)	1.01 (0.64-1.58)
(-)	(+)	31 (7.2)	72 (13.7)	33 (20.8)	2.04 (1.26-3.29)	2.93 (1.63-5.29)

B, 4th survey

		Age, years			Age, years	
HP	AG	60-69, n (%)	70-79, n (%)	≥80, n (%)	70-79, OR (95%CI)	≥80, OR (95% CI)
(-)	(-)	309 (56.2)	210 (49.1)	103 (46.2)	1 (Ref)	1 (Ref)
(+)	(-)	100 (18.2)	71 (16.6)	29 (13.0)	1.05 (0.74-1.48)	0.87 (0.54-1.39)
(+)	(+)	117 (21.3)	106 (24.8)	63 (28.3)	1.33 (0.97-1.83)	1.62 (1.11-2.36)
(-)	(+)	24 (4.4)	41 (9.6)	28 (12.6)	2.51 (1.48-4.28)	3.50 (1.94-6.31)

ORs of the subjects 70-79 years or ≥80 years were determined using logistic regression analysis. OR, odds ratio; CI, confidence interval; *HP, Helicobacter pylori*; AG, atrophic gastritis

Discussion

This is the first to estimate the prevalence and secular trends of AG with a 10-year interval in a large-scale cross-sectional study using data from population-based cohort in Japanese men and women. In this study, the prevalence of AG and *H. pylori* infection were significantly lower in the fourth survey than in the baseline survey. The prevalence of AG increased with age, whereas the prevalence of *H. pylori* infection increased with age except for the elderly group that showed an inverted U-shaped association. Stratified for age, the prevalence of AG and *H. pylori* infection were significantly lower in the fourth survey than at baseline, and the curve of the fourth survey shifted further to the right of the curve at baseline. Of a total of 1690 patients in the baseline study (864 mountain, 826 coastal), 927 (373 mountain, 554 coastal) also participated in the fourth study, therefore the follow-up rate for the same patients was 54.9% (43.2% in mountainous areas, 67.1% in coastal areas). The results in these follow-up participants also showed a similar trend to the analysis results for the entire cohort (Table SI).

AG is generally recognized as a precursor of gastric cancer (1), but there are few epidemiological studies using large population-based data on the prevalence of AG (35). As for the prevalence and secular trends of AG using serological test results based on population-based cohort data, there has been only one report by Song *et al* in Sweden using only PG I as an indicator of AG (25). As far as we know, this is the first report of the prevalence of AG and secular trends determined by PG test (PG I and I/II ratio) using population-based cohort data in Japan. Since serum PG tests can be measured easily, rapidly, at low cost, and with minimal invasiveness, this approach could be used for evaluating functional AG in a large population. PG I and the I/II ratio show different changes during the natural history of AG. PG I is known to increase from normal mucosa to non-atrophic *H. pylori*-related gastritis and then decreases as AG extends, so if only PG I is used as an indicator of gastric atrophy, low PG I would include not only those with AG but also those with normal gastric mucosa. The pepsinogen I/II ratio shows a continuous decrease during the process from normal gastric mucosa through non-atrophic gastritis to AG (36). Therefore, the PG test was used as an indicator of gastric atrophy to estimate the prevalence of AG in this population-based screening survey.

It is generally believed that differences in hygiene levels or opportunities for oral infection during infancy affect the prevalence of *H. pylori* infection (37). In developed countries, infection rates are reported to be about 10-20% and increase with age (8). The prevalence of *H. pylori* infection in Japan has been showed in a few large epidemiological studies. However, previous reports indicated the prevalence in the Japanese population at a time when eradication therapy for *H. pylori*-related gastritis was not generally undergone. In 2008-2010, the prevalence of *H. pylori* infection detected in urban inhabitants of Japan with a relatively high proportion of young participants was estimated and shown to increase with age (15). Moreover, the graphs showing the prevalence of *H. pylori* infection by age groups were clearly shifted to the right compared to previous studies (38,39). In the present study as well, the prevalence of *H. pylori* infection was significantly

lower in the fourth survey than at baseline; thus, the curve of the fourth survey shifted further to the right of the curve of the baseline survey. *H. pylori* is believed to disappear in the gastric mucosa with extensive atrophy (4). The general trend is that as gastric atrophy develops extensively due to the persistence of *H. pylori*-related gastritis, the number of *H. pylori* bacteria in the epithelium decreases gradually and finally the bacteria are completely expelled from the stomach, leading to the state of spontaneous eradication resulting in the disappearance of bacterium-specific serum antibodies (40,41). In addition, in elderly persons, it is unavoidable that the antibody titer decreases with aging, and contamination of eradicated cases is inevitable. In this study, the *HP* (-) AG (+) subgroup proportions were significantly higher in the 70-79 or ≥80 age groups compared with the 60-69 age group, at both baseline and fourth survey. Thus, the prevalence of *HP* (-) AG (+) tended to increase significantly with age in the elderly. However, in follow-up cases analysis, when *HP* (+) AG (+) in the baseline survey (n=140) were analysis target and the outcome was *HP* (-) AG (+) in the fourth survey, the estimated incidence compared with those aged 60-69 years was 0.82 (0.36 to 1.87) for age group 70-79 years and 0 for age group ≥80 years, respectively. Consequently, the trend towards a significantly lower prevalence of *HP* infection with age in the elderly did not appear to reflect spontaneous eradication of the bacteria as an end result of the progression of chronic atrophic gastritis, in the present analysis. The potential for increased natural eradication of bacteria with aging requires further analysis. As for autoimmune gastritis, the prevalence is low (0.49%) in Japan (42). Therefore, the possibility of autoimmune gastritis among the examined AG cases with *H. pylori*-negative in this study was considered negligible.

One of the limitations of the present study is that the diagnosis of *H. pylori* infection and AG was based on serological tests. However, if *H. pylori*-positive or AG-positive misclassification by the serological test occurs equally in all subjects, the risk of exposure misclassification was underestimated. Second, although the participant population of the present study consisted of a large number, these participants were recruited from only two regions, the mountainous and coastal regions, and thus may not be representative of the general population. In this study, we selected two regions (mountain and coast) located in the central and southern part of Wakayama Prefecture, which has a low population movement according to the Japanese census and is one of the regions with a high mortality risk of gastric cancer and colorectal cancer in Japan (27). The values of anthropometric factors (mean BMI values) of the participants in this study were not significantly different from those of the general Japanese population of the same age group. It is likely that the subject selection in this study did not cause significant differences from the general Japanese population of the same age group. Thus, the results of this study may be generalizable to Japanese populations in areas with high gastric and colorectal cancer risk. On the other hand, the proportions of current smokers and drinking habits in this study were lower than those of the general Japanese population, suggesting that the study subjects may have had healthier lifestyles. This selection bias should be considered when generalizing the results of this study. Third, this study may have a healthy user bias. Unhealthy individuals drop out and healthy individuals remain for follow-up. Therefore, the possibility of this bias should be considered when generalizing the results. However, in this study, the results in the follow-up participants also showed a similar trend to the analysis results for the entire data. The fourth limitation is that the progressive spread of *H. pylori* eradication therapy between 2005 and 2016 in Japan might have affected the results to some extent. According to the questionnaire at the time of the fourth survey, 131/1906 (6.9%) of the subjects had a history of *H. pylori* eradication therapy in the present study and we excluded these *H. pylori* eradicated subjects from the analysis. Therefore, *H. pylori* eradication therapy was likely to have had a limited impact on the present findings.

In conclusion, this population-based cross-sectional study with a 10-year interval survey using a large population clarified that the prevalence of AG and *H. pylori* infection decreased significantly. This change will probably contribute to decreasing the future trends in the prevalence of *H. pylori*-related diseases, including extra-gastric target organs.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

II, NY, TM and MI conceived and planned the present study. II, NY, TM, KM and MI analyzed and interpreted data. II drafted the manuscript. TI, CH, SM, HO, HK, TA, KN and ST made substantial contributions to the study design and protocol, data collection and screening, and revising the draft critically for important intellectual content. NY and MI edited

the final draft. II and NY confirm the authenticity of all the raw data. All authors read and approved the final version.

Ethics approval nand consent to participate

The study was conducted with the approval of the ethics committees of the University of Tokyo (approval nos. 1264 and 1326), Tokyo University of Marine Science and Technology (approval no. 187), and the University of Wakayama Medical University (approval no. 373). All participants provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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