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Prevalence of *Helicobacter pylori* infection in two Spanish regions with different incidence of gastric cancer

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Abstract. It is a cross-sectional study, comparing the prevalence of *Helicobacter pylori* infection (prevalence of IgG antibodies to *H. pylori*) in the healthy population of Ubrique and Grazalema (mountain location, mortality from stomach cancer 20/100,000) and in Barbate, (coastal location, mortality from stomach cancer 10/100,000) in the province of Cádiz, southern Spain. The subjects were randomly selected, 163 men and 169 women, 18 years or older; 179 persons were studied in the inland, and 154 in the littoral in January 1997. Of the 332 subjects investigated, 43% were positive, a mean antibody titer of 337 IU/l (95% CI: 254–420), and 56% were negative, with a mean titer of 18 IU/l (95% CI: 15–19). In the coastal population, 30% has positive titers and 54% in the mountain location. By age: 18–40 years, 30% of littoral and 41% of inland population had positive titers; 41–60 years, 35% of those living in the littoral and 58% of inland population had positive titers; > 60 years, 24% of coastal inhabitants and 62% of those living in the inland had positive titers. Living in mountain locations in the province of Cádiz involves a greater ecological risk for *H. pylori* infection ($p < 0.05$).

Key words: Altitude, Gastric cancer, Geographic location, Healthy subjects, *Helicobacter pylori* infection

Abbreviations: CI = confidence interval

Mortality due to stomach cancer in Spanish men and women has decreased steadily since 1963 [1]. However, the decline has been uneven in different areas, and pockets of high mortality remain in mountainous areas. In comparison with coastal areas of the Iberian Peninsula, mortality is much higher in the interior, especially in provinces with a mean altitude greater than 600 m above sea level [1].

In the province of Cádiz, overall standardized mortality rates are 27/100,000 for men and 10/100,000 for women. In the town of Barbate, located on the Atlantic coast, the standardized mortality rate for stomach cancer is 10/100,000. In the towns of Grazalema and Ubrique, located in a mountainous area 65 km inland [1, 2], standardized mortality rates for stomach cancer are 20/100,000 [3]. The province of Cádiz is therefore an excellent model for research on the impact of *Helicobacter pylori* infection on geographical differences in mortality. The relationship between *H. pylori* infection and stomach cancer [4–7] has been explained on the basis of the theory of gastric carcinogenesis proposed by Correa [8, 9].

For epidemiological purposes, infection by *H. pylori* can be studied indirectly by determining the titer of IgG antibodies. This assay is technically straightforward and shows good reproducibility [10, 11].

We investigated the relationship between differences in rates of *H. pylori* infection in coastal and mountain populations and mortality due to stomach cancer.

Materials and methods

In this observational, descriptive, cross-sectional study we compared *H. pylori* IgG antibody titers in inhabitants of Grazalema and Ubrique, two towns located in mountainous areas of the province, with titers in the inhabitants of Barbate, a coastal town 65 km away.

Population

The subjects were healthy men and women aged 18 years or older, residing in Ubrique (300 m above sea level) or Grazalema (900 m above sea level), with a total population between them of 20,000, and in the coastal town of Barbate, with a total population of 20,000. Mean standardized mortality due to stomach

cancer in the last 5 years was 20/100,000 in the mountain location and 10/100,000 in the coastal location [3]. All subjects were healthy and asymptomatic at the time of the study January 1997, and none had received prolonged antibiotic or antiacid treatment. We excluded all subjects with known chronic disease and all subjects with known addiction to intravenous drugs.

Sample, sampling method and setting

The sample was stratified into three groups according to age: 18–40 years, 41–60 years, and older than 60 years. At least 50 persons of each sex were included in each age group from both locations. The total sample consisted of 332 persons: 154 individuals from the coastal location (Barbate) and 179 from the mountain location (Grazalema and Ubrique).

Subjects were chosen randomly from among the three age groups and the populations in the two geographical locations (Table 1). The age groups were defined on the basis of the distribution of global mortality rates for stomach cancer in Spain. Of patients younger than 40 years, only 1.6% die of stomach cancer (low mortality); 15% die between the ages of 41 and 60 years (intermediate mortality), and 83% die after the age of 60 years (high mortality) [12].

The data for this populational study were obtained in the subjects' place of habitual residence. As the qualitative variable we used place of residence (mountain or coastal). All subjects included in the study were permanent residents of the location involved; emigration was rare in all three towns. Because of its flourishing leather processing industry, the general economic level was higher in Ubrique than in Grazalema (meat processing industry) or Barbate (seasonal tuna fishing).

As the quantitative variable we used serum titer of *H. pylori* IgG antibodies, measured by enzyme-linked immunosorbent assay with the Biolab Malakit assay (Biolab, S.A., Wavre, Belgium). The assay is based on the peroxidase-ABTS technique; the resulting green color was measured with a Labinstrument SLT-400 photometer (Salzburg, Austria) at 405 nm. This technique was found to provide meaningful results in determinations of *H. pylori* infection [13].

Statistical analysis

Descriptive analyses for all variables and inferential analyses for IgG serum titers, age group, sex, location were calculated with the Systat software package [14]. Confidence intervals (CI) were calculated according to the method of Gardner and Altman [15], and cut-off points were determined according to the recommendations of Strike [16].

Results

Overall results

Of the 332 subjects in the final sample, 43% (144/332) had positive titers of *H. pylori* IgG antibodies, and 56% (188/332) had negative titers. In positive cases the mean antibody titer was 337 IU/l (95% CI: 254–420), in negative cases the mean titer was 18 IU/l (95% CI: 15–19). The cut-off point of positive/negative values was 51 IU, with a sensitivity of 84% and a specificity of 99%.

Sex differences

We found no statistically significant differences between men and women in overall terms, nor were there differences between the sexes from the two locations or the three age groups ($p > 0.05$).

Age differences including both sexes

Positive titers were found in 36% (29/140) of the subjects aged 18–40 years, 47% (51/107) of those aged 41–60 years, and 46% (54/117) of those aged more than 60 years. There were no significant differences of serum positive cases between age groups; 41–60 and more than 60 years with respect to the total sample ($p > 0.05$); but there were significant differences between the age group of 18–40 years with respect to the total sample and with the other age groups ($p < 0.05$). We found no correlation between serum IgG antibody titer and age ($p > 0.05$). Mean serum titers did not differ significantly between the three age groups, although they did increase with age (18–41 years 114 IU/l, 41–60 years 157 IU/l, more than 60 years 195 IU/l).

Geographical differences

In the coastal location 30% of the inhabitants (46/153) has positive titers, in comparison with 54% (98/179) in the mountain location ($p < 0.05$). Mean serum antibody titer was 228 IU/l (95% CI: 96–359) in the former and 389 IU/l (95% CI: 185–495) in the latter.

Positive titers were found in 28% of the men (22/77) in the coastal location and in 50% of the men (43/86) in the mountain location ($p < 0.01$). Serum antibody titers were positive in 31% (24/76) of the women in the

Table 1. Distribution of sexes and age groups in samples from coastal and mountain locations in southern Spain in February, 1997

Location	Male/ Female	18–40 years	41–50 years	> 60 years
Coastal	77/153	52	51	50
Mountain	86/179	56	56	67
Total	163/169	108	107	117

coastal location and in 59% of the women (55/93) in the mountain location; this difference was also highly significant ($p < 0.001$).

The findings in different age groups differed clearly between inhabitants of the coastal and mountain locations (Table 2). Of the subjects aged 18–40 years, 30% (16/52) of those living in the coastal location and 41% (23/56) of those in the mountain location had positive titers ($p < 0.05$, Mann–Whitney test). Of those aged 41–60 years, 35% (18/51) of those living in the coastal location and 59% (33/56) of those in the mountain location had positive titers ($p > 0.05$, not significant). The difference was greatest in those aged more than 60 years: positive results were found in 24% (12/50) of those living in the coastal location and 62% (42/67) of those in the mountain location ($p < 0.01$).

Discussion

Serum titers of anti-*H. pylori* antibodies were highest in subjects older than 40 years residing in the mountain location. This group had the highest risk for stomach cancer, and had more frequent and more intense *H. pylori* infections, than inhabitants of the coastal location. The greatest difference in prevalence of *H. pylori* infection between coastal and mountain locations was found for subjects aged 41–60 years old.

A greater prevalence of *H. pylori* infection in the mountain than in the coastal location was reported in a Peruvian study by Cayetano [17]; in this case the author attributed the difference not to ecological factors, but to hygiene and health practices. Socioeconomic factors, especially overcrowded conditions during childhood, were also cited by Malaty and Graham [18]. The differences between coastal and mountain locations in southern Spain cannot be attributed to the potable water supply or the availability of refrigerators in the home, as reported by Boeing [19], as both locations have an excellent public water supply, and more homes in the mountain location than in the coastal location have refrigerators (personal observation).

Table 2. Number and percentage of subjects from different age groups from coastal and mountain locations with positive serum *Helicobacter pylori* IgG antibody titers

Location and age	N	Percent	Confidence Mean	interval	*p*-value
Coastal					
18–40 years	16	30.7	294.3	6–582	< 0.05
41–60 years	18	12.2	244.0	24–463	> 0.05
> 60 years	12	24.0	116.1	80–151	< 0.01
Mountain					
18–40 years	23	41.0	283.4	108–460	
41–60 years	33	58.9	347.2	116–578	
> 60 years	42	62.6	480.0	352–608	

The two locations studied here are relatively near each other (65 km), and the socioeconomic level at the time of the study was slightly higher in the mountain (Grazalema and Ubrique) than in the coastal location (Barbate). Our finding of a higher rate of infection in the location that enjoyed the higher socioeconomic level contrasts with the higher prevalences reported for economically depressed areas [20].

Our findings for the different age groups showed differences between the two locations: the prevalence of *H. pylori* infection was similar in all three age groups in the coastal location, but increased significantly with age in the mountain location. Several studies have reported an increase in the prevalence of infection with age in developed countries [4, 21–24], as we found once again for inhabitants of the mountainous location. No such increase, however, was detected in the coastal location, where the overall prevalence was lower. This finding supports the hypothesis that infection in persons more than 40 years old is an etiological factor related with the increased gastric carcinogenesis in persons of this age group living in inland locations.

Because of the cross-sectional nature of this study we cannot say whether the higher prevalence of infection in persons older than 60 years represents infections acquired at this age or the persistence of childhood infection. The latter possibility is supported by the findings of Mendal et al. [22], who reported that *H. pylori* infection is closely associated with childhood living conditions. In both of the locations we studied, the socioeconomic conditions during the subjects' childhood were much lower than at the time of the study. However, if the higher prevalence of *H. pylori* infection among persons older than 60 years in the mountain location represented the persistence of infections acquired during childhood, this would not account for the absence of age-related differences in prevalence in the coastal location, where previous socioeconomic conditions were similar to those in the mountain location. The difference between the two locations thus appears to be related with ecological factors that influence the prevalence of infection. The microaerophilic nature of *H. pylori* may allow this microorganism to survive better at higher altitudes than at sea level [17].

We have no information on possible differences in nutritional patterns at the two locations 20 to 40 years ago, which probably had a greater influence on prevalences than current dietary habits. However, we can

assume that salted and air-cured whole ham, a traditional product of mountain regions throughout much of southern Spain, was widely consumed in the past in Grazalema and Ubrique, whereas fish and seafood, particularly tuna, were more often consumed in the coastal location of Barbate. Higher mortality rates from gastric cancer in Spain [1] overlap the map of ham-producing areas, and we cannot rule out that high levels of anti-*H. pylori* IgG in pork and ham might have influenced our serological findings [25, 26].

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