Alimentary Tract

Prevalence, incidence and risk factors for *Helicobacter pylori* infection in a cohort of Portuguese adolescents (EpiTeen)

Joana Bastos^{a,b}, Bárbara Peleteiro^{a,b}, Hugo Pinto^{a,b}, Ana Marinho^c, João T. Guimarães^{b,c,d},
Elisabete Ramos^{a,b}, Carlo La Vecchia^{e,f}, Henrique Barros^{a,b}, Nuno Lunet^{a,b,*}

^a Department of Clinical Epidemiology, Predictive Medicine and Public Health, University of Porto Medical School, Porto, Portugal

^b Institute of Public Health of the University of Porto (ISPUP), Porto, Portugal

^c Department of Clinical Pathology Service, Hospital São João Medical Center, Porto, Portugal

^d Department of Biochemistry, University of Porto Medical School, Porto, Portugal

^e Department of Epidemiology, "Mario Negri" Institute for Pharmacologic Research, Milan, Italy

^f Department of Medical Sciences and Public Health, University of Milan, Milan, Italy

ARTICLE INFO

Article history:

Received 4 September 2012

Accepted 18 November 2012

Available online 21 December 2012

Keywords:

Adolescents

Helicobacter pylori

Incidence

Portugal

Prevalence

ABSTRACT

Background: *Helicobacter pylori* infection is acquired mainly during childhood, but it may occur throughout life. Understanding the determinants of infection at different ages is essential to clarify dynamics of *H. pylori* related diseases and to design preventive strategies.

Aim: To estimate the prevalence of *H. pylori* infection at the age of 13 and the incidence after a 3-year follow-up and to identify risk factors for infection.

Subjects and methods: Adolescents born in 1990 were recruited in schools from Porto. Whole-cell anti-*H. pylori* IgG antibodies were quantified by ELISA. Prevalence ratios (PR) and incidence rate ratios (RR) adjusted for parental education were computed at baseline ($n = 1312$) and at follow-up ($n = 280$).

Results: The prevalence was 66.2%, lower in subjects with more educated parents (PR = 0.72, 95%CI: 0.63–0.82), and higher for those having more than one sibling (PR = 1.10, 95%CI: 1.02–1.19) and for smokers (PR = 1.11, 95%CI: 1.02–1.20). The incidence was 4.1/100 person-years. Smoking (RR = 2.35, 95%CI: 1.16–4.75) and type of school (RR = 0.38, 95%CI: 0.16–0.95) were associated with the incidence of infection.

Conclusions: Prevalence and incidence of *H. pylori* infection were high, suggesting that gastric cancer will remain an important public health problem in this generation of Portuguese. We identified smoking as a modifiable risk factor for infection.

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1. Introduction

Half of the world adult population is infected by *Helicobacter pylori* [1], a human carcinogen [2] that is acquired mainly in childhood [3]. The prevalence varies appreciably across geographical areas [4,5] and is inversely associated with socioeconomic status or household hygiene and sanitation [6]. Over the last decades, the prevalence of infection in adult populations declined to below 50% in the more affluent countries of Europe [7–9], and this has contributed to the steep decline in gastric cancer incidence and mortality [10–12]. Despite the steady declines observed in Portugal since the 1970s [10,12,13], the country presented the highest

gastric cancer incidence (19.2 per 100 000 in males and 9.2 per 100 000 in females) and mortality rates (15.0 per 100 000 men, and 6.8 per 100 000 women) in the European Union (27 countries) in 2008 [14].

Age at acquisition of infection is an important determinant of the *H. pylori*-related gastric outcomes [15]. The birth-cohort pattern observed for gastric and duodenal ulcer could be explained by the decrease in the frequency of *H. pylori* infection accompanied by a simultaneous shift to acquisition at older ages [15]. High prevalence of infection at younger ages is associated with higher gastric cancer mortality than in settings with a similar frequency of infection in older subjects [16] but later acquisition.

Information on the incidence and prevalence of *H. pylori* infection and the understanding of its determinants at different ages may contribute to better understand the relation between this infection and the frequency of gastric cancer, ultimately supporting the definition of a life course framework for gastric pathology research and allowing the estimation of the future burden of gastric disease. Therefore, we aimed to quantify the prevalence of *H. pylori*

* Corresponding author at: Departamento de Epidemiologia Clínica, Medicina Preditiva e Saúde Pública, Faculdade de Medicina da Universidade do Porto, Al. Prof. Hernâni Monteiro, 4200-319 Porto, Portugal. Tel.: +351 225513652; fax: +351 225513653.

E-mail address: nlunet@med.up.pt (N. Lunet).

infection at the age of 13 and the incidence after a 3-year follow-up in a cohort of Portuguese adolescents, and to identify risk factors for infection at different ages.

2. Methods

A sample of adolescents born in 1990 was recruited in the schools from Porto, Portugal, in 2003/2004 (the EpiTeen cohort). School is compulsory at this age, and virtually all children are registered in a school of their dwelling area. A detailed description of the general selection procedures and participants' characteristics was published before [17,18]. In brief, the executive boards of all schools from Porto (27 public and 24 private) allowed the research team to contact the eligible students and their families. In compliant schools [all the public and 19 (79%) of the private], 2787 eligible adolescents were identified (2126 in public and 661 in private schools). We classified as refusals the eligible subjects who did not return the signed informed consent form ($n=583$, 20.9%). Those agreeing to participate were contacted at school: 44 students (1.6%) could not be reached during the evaluation period because were missing classes; the remaining 2160 (1651 public and 509 private school students) provided information at least for part of the planned assessment (Fig. 1). This resulted in a proportion of participants of 77.5%, similar in public and private schools (77.7% vs. 76.7%, $P=0.613$) [17].

During the baseline evaluation the participants answered two standardized questionnaires: one was completed by the adolescents at home, with the help of their legal guardians, to obtain data on social, demographic and behavioural characteristics as well as on individual and family history of disease; the other, used to collect information on health behaviours, particularly physical activity, smoking and alcohol consumption, was completed by the adolescents at school, during the visit of the research team. A physical examination was performed at school, between 8 a.m. and 10 a.m., by a team of experienced nurses, nutritionists and physicians, following standardized procedures. A 12-h overnight intravenous blood sample was obtained from 1390 participants. For the present study, we assessed the *H. pylori* status of 1312 subjects (94.4%) from which a large enough aliquot was available (Fig. 1).

During a follow-up conducted in 2007/2008, information regarding *H. pylori* infection was available for 280 (63.1%) participants without evidence of infection at the baseline evaluation (median follow-up: 37 months) (Fig. 1). A random sample, stratified by parental education, of approximately 10% of the participants with evidence of infection at baseline ($n=76$) was used to quantify the seroreversion rate (median time of follow-up: 39 months).

Serum samples were kept frozen at -80°C until analysis. Serum anti-*H. pylori* IgG titres were assessed by ELISA (Anti-*H. pylori* ELISA, EuroImmun, Lubeck, Germany). The participants' infection status was classified as negative if the antibody concentration was lower than 16 RU/ml, borderline if the antibody concentration was equal or higher than 16 RU/ml and lower than 22 RU/ml, and positive if the antibody concentration was 22 RU/ml or higher. For the current analysis, the adolescents with a borderline result (first evaluation: $n=22$; second evaluation: $n=6$) were considered infected.

2.1. Statistical analysis

For data analysis, parental education was defined as the highest educational level achieved by any one of the parents. The crowding index was calculated as the number of individuals living in the same house over the number of rooms; the cut point used to create a dichotomous variable was 1.3, which corresponds to the median in this population. The participants were classified as ever or never having tried smoking and alcohol consumption at the age of 13.

The history of antibiotics use was also assessed in the follow-up evaluation.

Proportions were compared using the χ^2 test, or Fisher's exact test, when appropriate. Binomial regression was used to estimate parental education-adjusted prevalence ratios (PR) and the corresponding 95% confidence intervals (CI). Poisson regression was used to compute parental education-adjusted incidence rate ratios (RR) and the corresponding 95%CI. The statistical analysis was conducted using STATA®, version 9.2.

2.2. Ethics

The study was approved by the Ethics Committee of Hospital S. João and written consent was obtained from both legal guardians and adolescents.

3. Results

3.1. Prevalence of *H. pylori* infection at the age of 13

At baseline, the prevalence of *H. pylori* infection was 66.2% (95%CI: 63.6–68.7%).

As shown in Table 1, there was an inverse association between infection and the mother's and father's education. The adolescents studying in private schools were less likely to be infected (private vs. public: PR=0.86, 95%CI: 0.78–0.96). The prevalence of infection was higher in subjects with more siblings (>1 vs. ≤ 1 : PR=1.12, 95%CI: 1.04–1.22), living in houses with a higher crowding index (>1.3 vs. ≤ 1.3 : PR=1.09, 95%CI: 1.01–1.19) or smokers (ever vs. never: PR=1.12, 95%CI: 1.03–1.22). No significant associations were found with gender, bathing habits or care in washing hands before handling food (Table 1).

After adjustment for parental education, the number of siblings (>1 vs. ≤ 1 : PR=1.10, 95%CI: 1.02–1.19) and tobacco consumption (ever vs. never: PR=1.11, 95%CI: 1.02–1.20) remained statistically significant (Table 1).

3.2. Incidence of *H. pylori* infection between the ages of 13 and 17

The incidence rate was 4.1/100 person-years (95%CI: 3.0–5.8).

A strong positive association was found between smoking at the age of 13 and incidence of *H. pylori* infection (ever vs. never: RR=2.34, 95%CI: 1.16–4.72), even after adjustment for parental education (ever vs. never: RR=2.35, 95%CI: 1.16–4.75). Studying in a public school was also a risk factor for the acquisition of *H. pylori* infection, independently from parental education, (private vs. public: RR=0.38, 95%CI: 0.16–0.95) (Table 2).

The seroreversion rate was 1.6/100 person-years (95%CI: 0.6–4.4).

4. Discussion

The present study allowed us to estimate the point prevalence and the incidence of *H. pylori* infection in a representative sample of urban adolescents. The main findings were that the incidence of *H. pylori* infection was high throughout adolescence, despite the already high prevalence observed at the baseline age of 13. Social class, represented by parental education, was a relevant determinant of the prevalence at baseline, and smoking was associated with the incidence of infection during adolescence.

The prevalence observed in this study was higher than reported for most European countries, and even many South American, African and Asian settings [19–22], where it ranged from less than 10% to 64%. The prevalence of infection was 69% in Vietnam, in adolescents aged between 10 and 14 years evaluated in 2005 [23], 78%

Fig. 1. Flow-chart describing the participants included in the analyses. *Participants without *H. pylori* testing had more educated parents (highest educational level achieved by any one of the parents – median number of school years: 11.0 vs. 10.0, $P=0.052$), but a similar sex distribution (males: 49.8% vs. 47.3%, $P=0.258$). †The participants' infection status was classified as negative if the antibody concentration was lower than 16 RU/ml, borderline if the antibody concentration was equal or higher than 16 RU/ml and lower than 22 RU/ml, and positive if the was 22 RU/ml or higher. ‡Participants with no information on *H. pylori* infection status at follow-up had less educated parents (median number of school years: 11.0 vs. 12.0, $P=0.032$), but there were no significant differences regarding gender (males: 52.4% vs. 50.0%, $P=0.620$).

in Rondônia, Brazil, in a sample of participants aged 11–20 years, evaluated in 2010 [24], and 82% in Iran, in subjects aged below 15 years, evaluated in 2006 [25]. In Portugal, the first national study addressing *H. pylori* prevalence (assessed by serology), published in 1994, showed that the prevalence of infection among children aged between 3 and 14 years was 46.2% [26]. In a recent study conducted in the south of Portugal, the prevalence of *H. pylori* infection (assessed by stool antigen test) in children aged between 3 and 14 years of age was 39.3%, and 51.5% in children aged between 11 and 15 years [27]. The prevalence found in our study is higher, which explains the higher gastric cancer incidence found in the North of Portugal [13,28].

Direct comparisons between studies assessing the frequency of *H. pylori* infection in adolescents is hampered by the diversity of age ranges, duration of follow-up, types of populations and characteristics of the diagnostic tools. The annual rates of seroconversion ranged from 0.1% to 1.9% in adolescents from high income countries, as assessed in the late 1990s [19]. In more recent studies, the annual rates were 1.5% in Japan [29], 1.4% in the USA [30], 2.5% in Turkey [31] and 7.0% in Turkish children living in Germany [31,32]. In a study from the South of Portugal, conducted between 2003 and 2006 it was 10.5/100 child-years in the age group 11–15 years [27], though only 52% participated in the follow-up study and from these 60% were lost during the 3-year follow-up.

A poor socio-economic condition favours infection, likely through poor sanitation, deficient hygiene and overcrowding [33–35]. We used education of the parents and type of school attended (private or public) as a surrogate measure of socio-economic status, and confirmed its importance as a determinant of infection. When including other potential markers of

socioeconomic status in the model, such as smoking and alcohol consumption, the results remained essentially unchanged (data not shown). The number of siblings and household overcrowding during childhood reflect the probability of close contacts with infected individuals and has also been associated with *H. pylori* infection [30,36–38]. Our results confirm the role of the number of siblings for the occurrence of infection in the childhood but not in adolescence. However, the interpretation of this result is limited by the fact that we were not able to measure *H. pylori* infection in the household members. The crowding index was based on the information collected at 13 years and it may not totally reflect the crowding conditions during early childhood, which may have contributed to the lack of association between this exposure and infection at the baseline; on the other hand, it is a measure of the crowding conditions in adolescence, and the lack of its association with the acquisition of infection is likely to reflect the more important effect of other factors than family close contacts for the acquisition of the infection at these ages.

We also evaluated bathing and hand-washing habits and hygiene in handling foods, but we found no associations with infection in these adolescents. A previous analysis of this dataset showed that the lowest hand washing frequency was associated with higher probability of diarrhoea occurrence [39], and therefore it is unlikely that the lack of association observed in the present investigation reflects underestimation due to social desirability bias [40].

Although controversial, there is a body of evidence regarding the relation between smoking and *H. pylori* infection [6]. The fact that tobacco smoking is associated with other lifestyles and with nutritional status [41–44] may contribute to the conflicting

Table 1Factors associated with the prevalence of *H. pylori* infection at the age of 13.

	N	<i>H. pylori</i> -infected, n (%)	Crude PR (95%CI)	Adjusted ^a PR (95%CI)
Mother's education ^b				
≤4 years	311	227 (72.3)	1.00	–
5–9 years	406	286 (70.4)	0.96 (0.88–1.06)	–
10–12 years	282	164 (58.2)	0.80 (0.71–0.90)	–
>12 years	254	141 (55.5)	0.76 (0.67–0.87)	–
Father's education ^b				
≤4 years	289	210 (72.7)	1.00	–
5–9 years	408	273 (66.9)	0.92 (0.83–1.02)	–
10–12 years	291	181 (62.2)	0.86 (0.76–0.96)	–
>12 years	215	112 (52.1)	0.72 (0.62–0.83)	–
Parental education ^b				
≤4 years	189	144 (76.2)	1.00	–
5–9 years	438	311 (71.0)	0.93 (0.84–1.03)	–
10–12 years	326	200 (61.4)	0.80 (0.72–0.90)	–
>12 years	310	170 (54.8)	0.72 (0.63–0.82)	–
Type of school				
Public	996	681 (68.4)	1.00	1.00
Private	316	187 (59.2)	0.86 (0.78–0.96)	0.93 (0.83–1.03)
Gender				
Females	691	473 (68.4)	1.00	1.00
Males	621	395 (63.6)	0.93 (0.86–1.00)	0.92 (0.85–1.00)
Number of siblings ^b				
≤1	892	564 (63.2)	1.00	1.00
>1	401	285 (71.1)	1.12 (1.04–1.22)	1.10 (1.02–1.19)
Crowding index ^b				
≤1.3	392	242 (61.7)	1.00	1.00
>1.3	872	587 (67.3)	1.09 (1.01–1.19)	1.05 (0.96–1.15)
Smoking ^b				
Never tried	958	612 (63.9)	1.00	1.00
Ever tried	308	220 (71.4)	1.12 (1.03–1.22)	1.11 (1.02–1.20)
Alcohol drinking ^b				
Never tried	575	390 (67.8)	1.00	1.00
Ever tried	688	438 (63.7)	0.94 (0.87–1.02)	0.96 (0.89–1.04)
Bath habits ^b				
Bath ≥4 times/week	1158	755 (65.2)	1.00	1.00
Bath <4 times/week	132	92 (69.7)	1.07 (0.95–1.21)	1.06 (0.94–1.19)
Washing hands before handling food ^b				
Always	913	604 (66.2)	1.00	1.00
Not always	379	244 (64.4)	0.97 (0.89–1.06)	1.02 (0.93–1.11)

PR, prevalence ratio; 95%CI, 95% confidence interval.

^a Adjusted for parental education (highest educational level achieved by any one of the parents).^b The sum of participants in each class is lower than the total ($n = 1312$) due to missing information.

findings on this topic. In our study, we opted for providing the PR and RR estimates adjusted only for parental education, because the point estimates did not vary meaningfully when BMI (above the 95th percentile, between the 85th and the 95th percentile and below the 85th percentile, as recommended for children and adolescents according to the age- and sex-specific BMI reference percentiles developed by the United States Centers for Disease Control and Prevention [45,46]) or dietary patterns were included in the model. This may reflect the fact that parental education is a surrogate for socioeconomic status and lifestyle exposures, but also the lack of association between each of the latter and *H. pylori* infection. In our study, after adjustment for parental education we did not find significant associations between BMI and *H. pylori* infection, (above the 95th percentile vs. below the 85th percentile: PR=0.92, 95%CI: 0.80–1.06; between the 85th and the 95th percentile vs. below the 85th percentile: PR=0.92, 95%CI: 0.75–1.12) or between dietary patterns (unpublished results) and infection (“High calorie” vs. “Healthy”: PR=1.01, 95%CI: 0.84–1.22; “Low calorie” vs. “Healthy”: PR=1.08, 95%CI: 0.93–1.25; “Undifferentiated” vs. “Healthy”: PR=0.96, 95%CI: 0.81–1.13). When including smoking habits in these models, the point estimates remained essentially unchanged. Although the association observed in our study between tobacco use and infection is relatively weak, it is biologically plausible and smoking was also shown to contribute for the failure of *H. pylori* eradication [47]. Our results show that there

is potential for intervention in the reduction of *H. pylori* infection during adolescence and this should be taken into account when estimating the potential benefits of tobacco control measures at early ages. A greater frequency of *H. pylori* infection in smokers may also at least in part contribute to explain the relation between tobacco and gastric cancer [12,48]. On the other hand, the lack of association between alcohol consumption and infection is in accordance with previous reports [6].

Our results show that nearly 16 in each 1000 *H. pylori* infected adolescents undergo seroreversion each year. In one of these adolescents a short course of antibiotics for respiratory disease had been prescribed a few months before the follow-up assessment, but no history of use of antibiotics was reported in the same period for the remaining. Serologic tests in children are not as accurate as in adults [49]; although using the cut-off values defined for adults may yield a lower sensitivity in infants, after the age of 12 years sensitivity and specificity become as high as in adults [49]. According to the manufacturer's description of the product, the test used to assess infection status in the present investigation has a sensitivity of 100% and a specificity of 94%, in adults. We classified the participants with borderline serum IgG titres as infected, but the results were not meaningfully different when participants with borderline results were considered not infected (data not shown). Furthermore, the declines in the antibody concentration of the participants in whom seroreversion was observed ranged between 72%

Table 2Factors associated with the incidence of *H. pylori* infection after the age of 13.

	N	Incident cases of infection	Person-years at risk	Crude RR (95%CI)	Adjusted ^a RR (95%CI)
Mother's education					
≤4 years	48	7	144	1.00	–
5–9 years	75	9	233	0.79 (0.30–2.13)	–
10–12 years	71	10	213	0.96 (0.37–2.54)	–
>12 years	86	9	256	0.72 (0.27–1.94)	–
Father's education ^b					
≤4 years	52	8	158	1.00	–
5–9 years	81	11	246	0.88 (0.35–2.20)	–
10–12 years	66	6	202	0.59 (0.20–1.69)	–
>12 years	77	10	226	0.87 (0.34–2.21)	–
Parental education					
≤4 years	31	5	92	1.00	–
5–9 years	74	8	232	0.63 (0.21–1.94)	–
10–12 years	70	9	213	0.78 (0.26–2.32)	–
>12 years	105	13	309	0.77 (0.28–2.17)	–
Type of school					
Public	190	29	570	1.00	1.00
Private	90	6	276	0.43 (0.18–1.03)	0.38 (0.16–0.95)
Gender					
Females	140	15	431	1.00	1.00
Males	140	20	415	1.38 (0.71–2.70)	1.39 (0.71–2.71)
Number of siblings					
≤1	210	23	635	1.00	1.00
>1	70	12	211	1.57 (0.78–3.16)	1.58 (0.78–3.17)
Crowding index ^b					
≤1.3	98	13	292	1.00	1.00
>1.3	178	21	542	0.87 (0.44–1.74)	0.88 (0.44–1.75)
Smoking ^b					
Never tried	218	22	664	1.00	1.00
Ever tried	53	12	155	2.34 (1.16–4.72)	2.35 (1.16–4.75)
Alcohol drinking ^b					
Never tried	112	16	341	1.00	1.00
Ever tried	164	19	492	0.82 (0.42–1.60)	0.82 (0.42–1.60)
Bath habits ^b					
Bath ≥4 times/week	250	30	757	1.00	1.00
Bath <4 times/week	29	5	86	1.47 (0.57–3.78)	1.47 (0.57–3.78)
Washing hands before handling food					
Always	186	24	569	1.00	1.00
Not always	94	11	277	0.94 (0.46–1.92)	0.94 (0.46–1.92)

RR, incidence rate ratio; 95%CI, 95% confidence interval.

^a Adjusted for parental education (highest educational level achieved by any one of the parents).^b The sum of participants in each class is lower than the total ($n = 280$) due to missing information.

and 99%, supporting the hypothesis of a previous infection that was resolved.

Two patterns of *H. pylori* prevalence in respect to age have been described. In middle and low income countries infection is acquired mainly in childhood and may reach nearly 100% during adulthood. In high income countries, the infection is less common in young children and gradually increases with age [5]. Portugal presents a very high prevalence of infection in adolescence, similarly to what is observed in middle and low income settings, but also a high incidence between the ages of 13 and 17, showing that the acquisition in this period is not negligible and contributes for the high prevalence later in life.

In conclusion, approximately two-thirds of these Portuguese 13 year-old were infected with *H. pylori*, and the incidence of infection remained high during the adolescence. Our results suggest that stomach cancer incidence and mortality in Portugal are likely to remain among the highest in Europe.

Conflict of interest statement

The authors declare that there are no conflicts of interest to disclose.

Sources of support

Grants from 'Fundação para a Ciência e a Tecnologia' (PTDC/SAU-ESA/103958/2008, PTDC/SAU-EPI/115254/2009, POCTI/SAU-ESP/62399/2004 and SFRH/BD/41504/2007) are gratefully

acknowledged. The work of CLV was supported by the 'Associazione Italiana per la Ricerca sul Cancro' (AIRC, Grant No. 10068). The authors report no other sources of founding.

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