Clinics and Research in Hepatology and Gastroenterology (2019) 43, 603—607

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com/en

ORIGINAL ARTICLE

Helicobacter pylori infection: association with dietary habits and socioeconomic conditions

Rosa Monno[a,*], Vittoriana De Laurentiis[a], Paolo Trerotoli[b], Anna Maria Roselli[a], Enzo Ierardi[c], Piero Portincasa[d]

[a] *Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari Aldo Moro, P. Giulio Cesare 70124 Bari, Italy*
[b] *Department of Biomedical Sciences and Human Oncology, University of Bari Aldo Moro, P. Giulio Cesare 70124 Bari, Italy*
[c] *Department of Emergency and Organ Transplantation University of Bari Aldo Moro, P. Giulio Cesare 70124 Bari, Italy*
[d] *Clinica Medica ''A. Murri'', Department of Biomedical Sciences and Human Oncology, University of Bari Aldo Moro, P. Giulio Cesare 70124 Bari, Italy*

Available online 21 March 2019

KEYWORDS
Food intake;
Living conditions;
Risk factors;
Urea breath test

Summary
Introduction: Few studies have investigated factors possibly related to the source of *Helicobacter pylori* infection in humans.
Materials and methods: This was a retrospective study including a population of 201 *H. pylori* positive patients and 259 H. pylori negative subjects observed at a tertiary referral center in Apulia. The *H. pylori* status was assessed by urea breath test. Data on socio-demographic characteristics and the consumption of different foods and beverages in the last year were collected by a questionnaire.
Results: No significant relationship was found between *H. pylori* infection and gender or age, type of employment, working in direct contact with the public, residence, level of education or exposure to pets. No association between *H. pylori* status and the consumption of fish, fruits, legumes, honey, spices, meats, milk and milk products including some typical product of our area was found. The same was true for the consumption of several kind of beverage including green tea and wine. Cigarette smoking and living in the same house with *H. pylori* positive relatives were significantly associated with H. pylori positivity. The intake of uncooked seafood (mussels and other molluscs) as well as some uncooked vegetables such as tomatos,pepper,and chicory,municipal water and the number of cups of coffee consumed per week correlated significantly with H. pylori status. The consumption of raw vegetables purchased from street vendors and the consumption of meals outside home were also associated with *H. pylori* infection.

* Corresponding author at: Department of Basic Medical Sciences, Neurosciences and Sense Organs. University of Bari Aldo Moro, Bari, Policlinico-P. Giulio Cesare 70124 Bari, Italy.
E-mail addresses: rosa.monno@uniba.it (R. Monno), vittoriana.delaurentiis@gmail.com (V. De Laurentiis), paolo.trerotoli@uniba.it (P. Trerotoli), annamros1@gmail.com (A.M. Roselli), ierardi.enzo@gmail.com (E. Ierardi), piero.portincasa@uniba.it (P. Portincasa).

https://doi.org/10.1016/j.clinre.2018.10.002
2210-7401/© 2018 Published by Elsevier Masson SAS.

Conclusions: Foods may represent an important route in the transmission of *H. pylori* among humans.
© 2018 Published by Elsevier Masson SAS.

Introduction

Helicobacter pylori is the cause of chronic gastritis, peptic ulcer disease, Mucosa-Associated Lymphoid Tissue (MALT lymphoma) and represents the major risk factor for the development of gastric cancer. The burden of *H. pylori* infection has been recently estimated in the Southern Italian Apulia region [1]. Direct person to person contact by either oral-oral or fecal-oral route is thought to be the most likely mode of transmission of *H. pylori* [2].

H. pylori antigens are commonly found in the stools of infected patients even if the isolation from human stools has rarely been reported. DNA of *H. pylori* is found in fecal samples of infected subjects [3,4]. Thus, the shedding of *H. pylori* with feces gives rise to the possibility that fecally contaminated water or foods could represent a vehicle of transmission.

H. pylori has been detected by molecular methods in several environmental matrices [5—13]. In the past *H. pylori* was never cultured from water or foods perhaps to its presence in these matrices in a viable but non-culturable state. Recently the in vitro isolation of *H. pylori* from water or food has been reported [5,9,10,13]. Therefore, *H. pylori* may be considered also as a food-borne pathogen. However, factors influencing the risk of acquisition of *H. pylori* infection are not yet fully established and few studies on the association of *H. pylori* infection with the possible risk factors affecting its acquisition have been reported. In this study, we investigated the association of *H. pylori* infection and demographic, socioeconomic conditions as well as dietary habits in our geographical area Table 1.

Subjects and methods

Study population

A total of 580 subjects (265 males and 315 females, age range 18-54 years) were consecutively enrolled at the Division of Internal Medicine, ''A. Murri'', University of Bari Medical School, Regional Hospital Policlinico, Italy within the screening procedure for *H. pylori* infection. All participants gave fully informed written consent and the protocol was approved by the local Ethics Committee of the Hospital.

Among the interviewed subjects, 120 were excluded because of faulty dietary information, leaving 460 evaluable individuals. Data were collected using a questionnaire completed in the presence of structured interviewers, and subjects were asked about their dietary habits in the previous twelve months. We excluded from the study subjects on vegetarian/vegan diets (Table 2.

The questionnaire required information on sociodemographic characteristics (age, gender, occupation, level of education, area of residence, size of household, contact with domestic animals, type of drinking water used, smoking habits and alcohol consumption). Their history of diseases was also recorded. The intake and the frequency (never, once/twice a month, twice/three times a week, more than three times a week) of the consumption of a wide range of food items was recorded including meat, fish, milk, cheeses, fruits, non-alcoholic and alcoholic beverages, legumes, spices and vegetables (Table 1.

The UBT was performed using the Breath Quality UBT-75 mg ^{13}C urea (AB Analitica SRL, Padova, Italy) in fasting subjects for at least 8 hrs and off of antibiotics in the prior month, and no proton pump inhibitors in the previous 2 weeks. Smoking and physical exercise were not allowed on the morning of the test. The sensitivity and specificity of UBT ranges from 88 to 95% and 95 to 100%, respectively and UBT samples were analyzed by an infrared mass spectrometry (HeliFANplus®, FAN GmbH, Leipzig, Germany). Delta values over baseline (DOB, δ%) were abnormal if > 4‰.

Statistical analysis

The sample size was determined to evaluate the hypothesis of observing a 10% difference in the proportion of consumption at least for one food three times between the *H. pylori* positive and negative groups. Given a type I error of 0.05 and a power of 80% the minimum sample size resulted 199 for each group.

All variables were categorized and summarized as mean and percentage, except age which was described as mean

Table 1 *H. pylori* UBT positivity by age.

Age (years)	*H. pylori*				*P*-value
	Positive	(%)	Negative	(%)	0.0518
≤ 20	8	3.98	25	9.65	
21—30	26	12.93	43	16.6	
31—50	73	36.32	81	31.28	
51—60	32	15.92	48	18.53	
> 60	62	30.85	62	23.94	
Total	201	100.00	259	100.00	

A value of $P < 0.05$ was considered to be significant.

Table 2 *H. pylori* UBT positivity by gender.

Gender	*H. pylori*				*P*-value
	Positive	(%)	Negative	(%)	0.7952
Male	80	39.8	100	38.61	
Female	121	60.2	159	61.39	
Total	201	100.00	259	100.00	

A value of $P < 0.05$ was considered to be significant.

Table 3 Comparison between *H. pylori* status and sociodemographic characteristics.

Gender	*H. pylori*				*P*-value
	Positive	(%)	Negative	(%)	0.7952
Male	80	39.8	100	38.61	
Female	121	60.2	159	61.39	
Total	201	100.00	259	100.00	

A value of $P < 0.05$ was considered to be significant.

and range. The chi-square test for independent samples was performed to evaluate the difference of percentage or the association between categorical variables (such as class of food consumption) and positivity to H. pylori. When appropriate, the difference of proportion between independent groups was analyzed through a Fisher Exact test. A *P*-value < 0.05 was considered as statistically significant.

Results

Of the 460 evaluable subjects 180 (39.1%) were males and 280 (60.9%) females with a mean age of 47.5 years (range: 18—64 years); 201 subjects were *H. pylori* positive (43.7%) and 259 subjects were *H. pylori* negative (56.3%). In the *H. pylori* positive group 80 (39.8%) were males and 121 (60.2%) were females. The mean age was 50.37 years (range 32—51 years) in the *H. pylori* positive group and 46.10 years (range 38—52 years) in the *H. pylori* negative subjects.

There was no significant difference between *H. pylori* positive and negative groups related to age class ($P = 0.0518$) and gender ($P = 0.7952$) (Table 1 and Table 2, respectively).

Comparison between *H. pylori* status and sociodemographic characteristics is reported in Table 3. No significant difference was observed between *H. pylori* positive and *H. pylori* negative subjects in terms of employment, working in direct contact with the public, residence (urban or rural), level of education and exposure to pets. However, reporting a family member with *H. pylori* infection living in the same house was significantly associated to *H. pylori* infection ($P = 0.0264$). A significant association was found also between *H. pylori* infection and smoking status ($P = 0.0152$) but no relationship existed between increasing percentages of *H. pylori* positive patients and increasing number of smoked cigarettes.

No association was found between *H. pylori* infection and frequency of consumption of several cooked, uncooked or preserved meats. The consumption of several types of fish (both cooked and raw) was not associated with *H. pylori* infection. Only the eating of raw molluscs and mussels was significantly associated with *H. pylori* ($P = 0.0108$ and 0.0067, respectively). The consumption of dairy products was also investigated, but neither milk nor milk products, that is fresh and matured cheeses (including some typical non-industrial products of Apulia such as ''mozzarella'' and ''ricotta'') correlated with *H. pylori* infection. The consumption of several kind of beverage, such as fresh and preserved fruit juices, including orange, lemon, lime, blueberry and pineapple did not appear to be associated with *H. pylori* infection. A similar result was observed for the consumption of green tea, wine and several items of peeled or not-peeled fruits. No association between *H. pylori* positivity and the consumption of other foods (legumes, honey) and spices (including garlic and chili pepper) was found in our study.

Instead, a statistically significant association existed between *H. pylori* infection and frequent intake (> 3 times/week) of raw vegetables, especially tomatoes ($P = 0.0426$), peppers ($P = 0.0155$) and chicory ($P = 0.0041$).

There was a statistically significant association between *H. pylori* positivity and the consumption of municipal water in comparison to the consumption of bottled mineral water ($P = 0.0439$). Also the intake and number of cup of coffee per week was statistically related to *H. pylori* infection ($P = 0.0022$).

Similarly, the consumption of food from street vendors and of meals outside the home was significantly associated with *H. pylori* infection when compared to subjects consuming foods purchased from the supermarket or prepared at home ($P < 0.0001$ and 0.0144, respectively).

Discussion

Person to person spreading is considered a major transmission route of *H. pylori*. Higher prevalence of *H. pylori* infection is related to low social class and high density living conditions. On these bases we performed a retrospective study on *H. pylori* positive and negative subjects.

Our results showed that the presence of *H. pylori* positive relative living in the same home represented a significant risk factor for *H. pylori* infection. In our study smoking was also significantly associated with *H. pylori* infection though in other studies the reported association between *H. pylori* infection and smoking is controversial. It has been found that prior smokers and current cigarette smokers are at a higher risk of *H. pylori* infection than subjects who never smoked [14]. These results have not been confirmed by other studies [15,16]. Finally, the recent report that smoking was found to significantly increase the likelihood of unsuccessful first-line treatment for *H. pylori* infection further accentuates the controversy over smoking as a factor capable of interfering not only with infection, but also with its therapeutic outcome [17].

In this study, the consumption of several types of meat as well as cured meats was not associated with *H. pylori* infection as well as there being no significant association between *H. pylori* positivity and raw and cooked fish consumption. Instead, the consumption of raw molluscs and mussels was significantly associated to *H. pylori* infection. In our area, Apulia, Southern Italy, this habit is very widespread. Hepatitis A virus (HAV) and other infections due to microorganisms transmitted via the fecal-oral route such as *Salmonella* are endemic in our area and, though not recently, a few cases of cholera have also been reported, *Vibrio cholerae* being isolated from some samples of mussels and raw vegetables. To support the role of the *H. pylori* transmission by the fecal-oral route, a substantial agreement between *H. pylori* infection and HAV seropositivity, typhoid fever and the presence of *Giardia* in stools has been previously reported [2,18] despite these data are not confirmed by the results of a systematic review and the question is still open [19].

No significant relation was found between the consumption of milk, yogurt and fresh milk products as fresh ''mozzarella and ricotta'' , often prepared in our area with unpasteurized milk. *H. pylori* has been detected by PCR in raw milk samples, in some commercial pasteurized milk samples and in more than 22% of milk and meat samples of sheep [20]. *H. pylori* isolation from milk and tissue samples from sheep have been reported [21]. In our study, the consumption of some type of vegetables such as tomatoes, peppers and chicory, is associated with *H. pylori* infection. The consumption of uncooked vegetables often irrigated with untreated sewage waters have also been implicated in *H. pylori* acquisition [22]. *H. pylori* is able to form biofilm, to reside as microcolonies on vegetable surfaces and so have the ability to survive on vegetables, cabbage in particular, for an extended period of time, suggesting that vegetables may serve as a mode of transmission [23].

In our area, the consumption of uncooked vegetables is frequent, and they are, in some occasions, fertilized with human and animal feces. The association between the consumption of uncooked vegetables and *H. pylori* infection may be another point in favor of transmission by the fecal-oral route. HAV and other infective agents transmitted via the fecal-oral route, such as *Salmonella*, are endemic in our area where small episodes of verocytotoxin-producing *Escherichia coli* (VTEC) infection has also been reported even if the VTEC source was not fully identified [24,25]. Other studies have not confirmed these data even if the isolation of *H. pylori* from the 13.45% of ready to eat foods such as salads and soups further supports the role of food in the transmission of this bacterium [13].

The consumption of fruits (peeled or not) was not associated with *H. pylori* infection. If this is due to the antioxidants and protective effect of vitamin C or D present in the fruits remains to be established. However, eating food from street vendors seems to be associated to an increased prevalence of *H. pylori* infection. This was also found in our study which demonstrates a significant association between the intake of food from street vendors and *H. pylori* in comparison of subjects consuming food prepared at home. This could be related to low hygiene conditions such as the exposure of food to contaminated water or soil. Food handling may contribute to contamination of food by hands, fingernails and oral secretions. *H. pylori* has been detected by molecular methods in the oral cavity and fingernails of *H. pylori* positive subjects [26]. Recent studies have raised the possibility that source of the water supply could be associated with seropositivity for *H. pylori* both in developing and developed countries. The DNA of *H. pylori* was detected in wastewater samples of river water, sea water, well water, drinking water and mineral water [5,7,27,28].

Recently *H. pylori* has been cultured in vitro from 3% of drinking water samples in Iran and isolated and genotyped also from about 2% of bottled mineral water [10]. In our study a significant correlation was found between *H. pylori* positivity and consumption of municipal water in comparison with subjects consuming only bottled mineral water.

It has demonstrated that alcohol consumption reduces the *H. pylori* infection rate and a moderate consumption may facilitate spontaneous elimination of *H. pylori* [29], [30]. Alcohol drinkers had 25% lower odds of being *H. pylori*-positive than non-drinkers [2]. Alcohol possesses strong antimicrobial activity and stimulates gastric acid secretion. In particular, red wine is able to prevent *H. pylori*—induced gastritis in a mouse model [31]. These data are not confirmed by a study involving 705 residents in the rural town of Cirò, a wine producing centre in Southern Italy. The seroprevalence of infection was slightly higher in wine drinkers (70%) than in non-drinkers and was less in subjects who reported a lifetime high alcohol consumption, even if these differences were statistically not significant. Therefore, wine consumption was not a protective factor against *H. pylori* infection [32]. These results agree with what observed in our study. We also showed that the frequency of coffee drinking increase the *H. pylori* infection rate, but this is not confirmed by other studies.

Conclusions

Dietary intake of some items of food and beverages may influence the acquisition of *H. pylori*. Further studies will offer new insight on the role of alimentary practices on *H. pylori* infection. The detection of DNA of *H. pylori and/or* the isolation of *H. pylori* from foods, water and other environmental matrices may help to identify the reservoir of this microorganism, opening new scenarios on the epidemiology and route of transmission of this bacterium. Also, studies on the antibacterial activity against *H. pylori* of some natural products including virgin olive oil may contribute to the exploration of new alternatives to traditional therapies so reducing the use of antibiotics [33]. In this way, the emergence of resistant strains may be avoided, reducing the incidence of gastric inflammation and associated disorders such as gastric cancer. Therefore this area of research is worthy of further investigations.

Funding support

This study was funded by a grant from the University of Bari ''Aldo Moro'', Bari, Italy. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Monno R, Portincasa P, Ierardi E, conceived and designed the work

De Laurentiis V, Roselli AM, collected the data

Trerotoli P, analyzed the data.

All authors reviewed and participated in amending the manuscript before submission of the mutually agreed final version of the manuscript.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Di Ciaula A, Scaccianoce G, Venerito M, et al. Eradication rates in Italian subjects heterogeneously managed for *Helicobacter*

pylori infection. Time to abandon empiric treatments in Southern Europe. JGLD 2017;26:129—37.
[2] Bui D, Brown HE, Harris RB, Oren E. Serologic Evidence for Fecal-Oral Transmission of *Helicobacter pylori*. Am J Trop Med Hyg 2016;94:82—8.
[3] Ierardi E, Margiotta M, Monno R, et al. A new semiquantitative method of quantifying *Helicobacter pylori* in stools. J Clin Gastroenterol 2002;35:375—8.
[4] Giorgio F, Ierardi E, Sorrentino C, et al. *Helicobacter pylori* DNA isolation in the stool: an essential pre-requisite for bacterial noninvasive molecular analysis. Scand J Gastroenterol 2016;51:1429—32.
[5] Lu Y, Redlinger TE, Avitia R, Galindo A, Goodman K. Isolation and genotyping of *Helicobacter pylori* from untreated municipal wastewater. Appl Environ Microbiol 2002;68:1436—9.
[6] Santiago P, Moreno Y, Ferrús MA. Identification of Viable *Helicobacter pylori* in Drinking Water Supplies by Cultural and Molecular Techniques. Helicobacter 2015;20:252—9.
[7] Queralt N, Bartolomé R, Araujo R. Detection of *Helicobacter pylori* DNA in human faeces and water with different levels of faecal pollution in the north-east of Spain. J Appl Microbiol 2005;98:889—95.
[8] Vale FF, Vítor JM. Transmission pathway of *Helicobacter pylori*: does food play a role in rural and urban areas? Int J Food Microbiol 2010;138:1—12.
[9] Ranjbar R, Khamesipour F, Jonaidi-Jafari N, Rahimi E. *Helicobacter pylori* isolated from Iranian drinking water: vacA, cagA, iceA, oipA and babA2 genotype status and antimicrobial resistance properties. FEBS Open Bio 2016;6:433—641.
[10] Ranjbar R, Khamesipour F, Jonaidi-Jafari N, Rahimi E. *Helicobacter pylori* in bottled mineral water: genotyping and antimicrobial resistance properties. BMC Microbiol 2016;16:40.
[11] Yahaghi E, Khamesipour F, Mashayekhi F, et al. *Helicobacter pylori* in vegetables and salads: genotyping and antimicrobial resistance properties. Biomed Res Int 2014 2014:757—941.
[12] Safaei HG, Rahimi E, Zandi A, Rashidipour A. *Helicobacter pylori* as a zoonotic infection: the detection of H. pylori antigens in the milk and faeces of cows. J Res Med Sci 2011;16:184—7.
[13] Hemmatinezhad B, Momtaz H, Rahimi E. VacA, cagA, iceA and oipA genotypes status and antimicrobial resistance properties of *Helicobacter pylori* isolated from various types of ready to eat foods. Ann Clin Microbiol Antimicrob 2016;15:2.
[14] Ghosh P, Bodhankar SL. Association of smoking, alcohol and NSAIDs use with expression of cag A and cag T genes of *Helicobacter pylori* in salivary samples of asymptomatic subjects. Asian Pac J Trop Biomed 2012;2:479—84.
[15] Yordanov D, Boyanova L, Markovska R, et al. Influence of Dietary Factors on *Helicobacter pylori* and CagA Seroprevalence in Bulgaria. Gastroenterol Res Pract 2017 2017;92(1):21—43.
[16] Seid A, Demsiss W. Feco-prevalence and risk factors of *Helicobacter pylori* infection among symptomatic patients at Dessie Referral Hospital. Ethiopia. BMC Infect Dis 2018;18(1):260.
[17] Itskoviz D, Boltin D, Leibovitzh H, et al. Smoking increases the likelihood of *Helicobacter pylori* treatment failure. Dig Liver Dis 2017;49:764—8.
[18] Monno R, Volpe A, Basho M, et al. *Helicobacter pylori* seroprevalence in selected groups of Albanian volunteers. Infection 2008;36:345—50.
[19] BinSaeed AA. Is there a link between seropositivity to *Helicobacter pylori* and hepatitis A virus? A systematic review. Int J Infect Dis 2010;14:e567—7.
[20] Saeidi E, Sheikhshahrokh A. vacA Genotype Status of *Helicobacter pylori* Isolated from Foods with Animal Origin. Biomed Res Int 2016;2016:8701067 [Epub 2016/03/07].
[21] Dore MP, Sepulveda AR, El-Zimaity H, et al. Isolation of *Helicobacter pylori* from sheep-implications for transmission to humans. Am J Gastroenterol 2001;96:1396—401.
[22] Hopkins RJ, Vial PA, Ferreccio C, et al. Seroprevalence of *Helicobacter pylori* in Chile: vegetables may serve as one route of transmission. J Infect Dis 1993;168:222—6.
[23] Ng CG, Loke MF, Goh KL, Vadivelu J, Ho B. Biofilm formation enhances *Helicobacter pylori* survivability in vegetables. Food Microbiol 2017;62:68—76.
[24] Monno R, Parisi A, Montagna C, et al. Comparison of two methods for the detection of verotoxin producing *E. coli* in human faecal samples during an outbreak of HUS in Apulia, Southern Italy. New Microbiol 2015;38:277—80.
[25] Germinario C, Caprioli A, Giordano M, et al. Community-wide outbreak of haemolytic uraemic syndrome associated with Shiga toxin 2-producing *Escherichia coli* O26:H11 in Southern Italy, summer 2013. Euro Surveill 2016.
[26] Dowsett SA, Archila L, Segreto VA, Gonzalez CR, Silva A, Vastola KA, et al. *Helicobacter pylori* Infection in Indigenous Families of Central America: Serostatus and Oral and Fingernail Carriage. J Clin Microbiol 1999;37(8):2456—60.
[27] Bahrami AR, Rahimi E, Ghasemian Safaei H. Detection of *Helicobacter pylori* in city water, dental units' water, and bottled mineral water in Isfahan, Iran. Sci World J 2013;2013:280—510, http://dx.doi.org/10.1155/2013/280510.
[28] Baker KH, Hegarty JP. Presence of *Helicobacter pylori* in drinking water is associated with clinical infection. Scand J Infect Dis 2001;33:744—6.
[29] Brenner H, Berg G, Lappus N, Kliebsch U, Bode G, Boeing H. Relation of smoking and alcohol and coffee consumption to active *Helicobacter pylori* infection: cross sectional study. Epidemiology 1999;10:214—8.
[30] Murray LJ, Lane AJ, Harvey IM, Donovan JL, Nair P, Harvey RF. Inverse relationship between alcohol consumption and active *Helicobacter pylori* infection: the Bristol Helicobacter project. Am J Gastroenterol 2002;97:2750—5.
[31] Ruggiero P, Rossi G, Tombola F, et al. Red wine and green tea reduce *H. pylori*- or VacA-induced gastritis in a mouse model. World J Gastroenterol 2007;13:349—54.
[32] Luzza F, Imeneo M, Maletta M, Pallone F. Smoking, alcohol and coffee consumption, and *H pylori* infection. Alcohol consumption eliminates rather than prevents infection with *H pylori*. BMJ 1998;316:1019.
[33] Shmuely H, Domniz N, Yahav J. Non-pharmacological treatment of *Helicobacter pylori*. World J Gastrointest Pharmacol Ther 2016;7:171—8.