

The association between *Helicobacter pylori* infection and food intolerance in western China

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Abstract

Introduction

Helicobacter pylori (*H. pylori*) has been found to be associated with extragastrointestinal diseases while the studies on its association with food intolerance are rare. In our study, we tried to explore the relationship between *H. pylori* infection and food intolerance.

Methods

We retrospectively analyzed the physical examination data of 21,822 subjects from February 2014 to December 2018 in this study. *H. pylori* infection was determined by ^{13}C urea breath test. The food specific IgG in serum were detected to assess the food tolerance status of egg, milk and wheat.

Results

The total infection rate of *H. pylori* was 39.3% and the total food intolerance rates of egg, milk and wheat were 25.2%, 9.0% and 4.7%, respectively. The infection rate of *H. pylori* was higher in males than that in females, while the intolerance rates were lower in males than that in females. The infection rates of *H. pylori* increased with age except for people over sixty, while the intolerance rates decreased. In *H. pylori* positive group, the intolerance rates were lower than that in the *H. pylori* negative group. At the same level of food intolerance, the intolerance rates in *H. pylori* positive group were lower than that in *H. pylori* negative group. Multivariate Logistic regression analysis showed that *H. pylori* infection was negatively correlated with intolerance of egg, milk and wheat (*OR* value of egg 0.844–0.873, milk 0.741–0.751, wheat 0.762–0.801).

Conclusions

H. pylori infection was negatively correlated with intolerance of egg, milk and wheat in Sichuan, western China.

Introduction

Helicobacter pylori (*H. pylori*) infection, which is considered as one of the main pathogenic factors of chronic gastritis, gastric ulcer and gastric cancer, is an important public health issue in the world ¹. However, growing evidences suggest that *H. pylori* infection affects not only the gastrointestinal tract but also extragastrointestinal function, which has become a research hotspot. Contrary to the traditional view that *H. pylori* is a risk factor for disease, some studies have found a negative correlation between *H. pylori* infection and the development of certain diseases. For example, *H. pylori* infection showed a negative correlation with the development of allergic diseases such as asthma and eosinophilic esophagitis, especially in children and young people with early allergic reactions ².

Food intolerance is the body's discomfort reaction to the food or food components. The mechanism is closely related to the body's immune system, reflecting the body's immune tolerance to food components. Food allergy has the similar pathogenesis, symptoms to food intolerance. Only a few studies have researched the relationship between *H. pylori* infection and food allergy, however, the results are controversial³. Fewer studies discuss directly on the relationship between *H. pylori* infection and food intolerance. The study of 12,765 people in northern China by Sai et al. suggested that crab intolerance may be related to *H. pylori* infection⁴.

In China, food intolerance may be affected by various social economy, eating habits, food types, geographical climate and so on⁵. Our study focused on the relationship between food types and food intolerance. The three types of food, egg, milk and wheat, are widely consumed in Sichuan area with a relatively high rate of intolerance. In this study, we use the three food to explore the association between *H. pylori* infection and food intolerance in Sichuan.

Results

Baseline of the study population characteristics. The demographic and laboratory baseline characteristics of 21,822 subjects (12,396 males and 9,426 females) were shown in Table 1. The average age was 43.82 ± 10.98 years (range 18-89 years). The total infection rate of *H. pylori* was 39.3% and the total intolerance rates of egg, milk and wheat were 25.2%, 9.0% and 4.7%, respectively. The infection rate of *H. pylori* was higher in males than that in females (39.9% vs. 38.6%, $P = 0.043$). The total intolerance rates of the three foods in males were all significantly lower than that in females (20.4% vs. 31.5% for egg, 7.9% vs. 10.5% for milk, 3.6% vs. 6.2% for wheat, all $P < 0.001$).

The subjects were further stratified by age to investigate the prevalence of *H. pylori* infection and intolerance rates of the three food. The results showed that the prevalence of *H. pylori* infection increased with age except those who over sixty, while the intolerance rates of all the three foods decreased with age. As showed in Table 2.

Food intolerance status. The total intolerance rates of egg, milk and wheat decreased in turn, which were 25.2%, 9.0% and 4.7% respectively. When divided by the three different levels, the rates of mild, moderate and severe intolerance were 12.3%, 8.1%, 4.7% for egg, 5.0%, 2.7%, 1.3% for milk, 3.5%, 1.1%, 0.2% for wheat, respectively. The food intolerance status of the three foods as shown in Figure 1.

Comparison of food intolerance in different *H. pylori* infection situation. The total intolerance of three foods in *H. pylori* positive group were all statistically lower than that in *H. pylori* negative group (22.8% vs. 26.7% for egg, 7.4% vs. 10.1% for milk, 3.9% vs. 5.3% for wheat). For all the three food intolerance levels, the intolerance rates showed similarly trends, which were all lower in the *H. pylori* positive groups than that in the *H. pylori* negative groups, see Figure 2.

Logistic regression analysis of *H. pylori* infection and food intolerance. Logistic regression analysis was performed to explore the independent association between *H. pylori* infection and food intolerance. In

univariate analysis, results showed that *H. pylori* infection was associated with lower risk of food intolerance ($OR = 0.814, P < 0.001$ for egg; $OR = 0.714, P < 0.001$ for milk; $OR = 0.732, P < 0.001$ for wheat, model 1). After adjusting for age and sex, the results remained significant ($OR = 0.844, P < 0.001$ for egg; $OR = 0.741, P < 0.001$ for milk; $OR = 0.762, P < 0.001$ for wheat, model 2). And further after adjusting BMI, HbA1c, total cholesterol, triglycerides, smoking, drinking, the OR for food intolerance also remained significant ($OR = 0.873, P < 0.001$ for egg; $OR = 0.751, P < 0.001$ for milk; $OR = 0.801, P = 0.003$ for wheat, model 3). As showed in Table 3.

Discussion

The infection rate of *H. pylori* was high all over the world, with 50% in China⁶. However, compared with the high *H. pylori* infection rate, only 15%-20% of the infected subjects had peptic ulcer, 5%-10% had *H. pylori*-related dyspepsia, about 1% had gastric cancer, mucosa-associated lymphoid tissue (MALT) lymphoma and other gastric malignant tumors⁷⁻⁹. Most of the infected subjects were asymptomatic and did not receive drug treatment. Scholars focused to explore the chronic process in such a large number of asymptomatic carriers. Meanwhile, it had been found out that the influence of *H. pylori* infection was not limited to the gastrointestinal tract itself. In 1994, Mendall et al. first reported the relationship between *H. pylori* infection and extragastric diseases¹⁰. Subsequently, neurological, cardiovascular, hematologic, dermatological, ocular, metabolic and allergic diseases were found to be associated to *H. pylori* infection¹¹. It was reported that immune mechanism may play an important role in the relationship between *H. pylori* infection and extragastric diseases¹². In consideration of the high *H. pylori* infection rate, the relationship between *H. pylori* and many other extragastric diseases can't be ignored.

Food intolerance was a chronic disease with many extragastric clinical manifestations which affected 15%-20% of the population⁵. The mechanism of food intolerance was multifactorial, which was related to digestive system factors such as food composition, metabolic enzyme activity, transport mechanism and intestinal permeability change. Food substances entered the circulation through digestion and induced the production of food-specific IgG¹³. Specific IgG antibodies corresponding to food could be detected in the serum of patients¹⁴. IgG antibody formed circulating immune complex with food particles through antigen-antibody reaction, which deposited in various organs or systems along with blood circulation. The high prevalence and chronic process of food intolerance as well as its relationship with digestive system and immune system was similar to *H. pylori* infection, which made us interested in exploring the correlation between them.

So far, studies on the relationship between food intolerance and *H. pylori* infection in large samples were limited. In our study, we analyzed the physical examination data of more than 20,000 subjects. We selected egg, milk and wheat as the research objects of food intolerance, which were commonly consumed in Sichuan area with a relatively high incidence of food intolerance. The results showed that the total infection rate of *H. pylori* was 39.3% and the total intolerance rates of egg, milk and wheat were 25.2%, 9.0% and 4.7%, respectively. The infection rate of *H. pylori* was higher in males than in females.

However, in comparison with females, males had significantly lower intolerance rates of all the three foods. With the increasing of age, the infection rates of *H. pylori* increased significantly except those who over sixty, meanwhile, the intolerance rates of the three foods all decreased with age. Furthermore, we stratified the subjects by *H. pylori* infection status. The intolerance rates of total or different intolerant levels in *H. pylori* positive group were all lower than that in *H. pylori* negative group. Univariate logistic regression analysis showed that *H. pylori* infection was associated with lower risk of food intolerance. After adjusting the confounding factors including sex, age, BMI, HbA1c, total cholesterol, triglycerides, smoking and drinking, the association between *H. pylori* infection and food intolerance remained statistically significant. All the above results suggested that *H. pylori* infection seemed to help the body to be tolerant to eggs, milk and wheat. Interestingly, our results were contrary to the similar study in China⁴. The differences might be related to the sample size, food types and geographical differences which needed to be further studied.

Previous studies had found that *H. pylori* infection affected immune regulation, so that *H. pylori* could avoid immune surveillance to get long-term colonization. This may also be the cause of its association with some extragastrointestinal diseases¹⁵. For example, the asthma had been reported an inverse association with *H. pylori* infection. The protective effects of *H. pylori* depended on Foxp3+ regulatory T cell (Treg)¹⁶. Treg was a potently immunosuppressive CD4+ T cell subset and played a key role in immune tolerance by controlling the extent of response to self and non-self antigens. It could promote the rapid recovery of immune homeostasis¹⁷. *H. pylori* also upregulated the expression of CD80 and IL-10 via TLRs on B-lymphocytes, and then promote Treg cell differentiation¹⁸. Idiopathic thrombocytopenic purpura (ITP), an autoimmune disorder which was been found association with *H. pylori* infection since 1999¹⁹. One of the mechanisms was the monocytes from *H. pylori* infection patients had an enhanced phagocytic capacity and low levels of inhibitory FcγRIIB, leading to increased monocyte function of autoreactivity with B and T lymphocytes. This may cause the production of autoantibodies by B-lymphocyte against circulating platelets¹². Therefore, *H. pylori* might be related to some extragastrointestinal diseases both through cellular or humoral immunity regulation. The symptoms of food intolerance were mainly caused by humoral immunity mediated by IgG. Future research on humoral immunity might be helpful to understand the correlation between *H. pylori* infection and food intolerance.

The limitation of our study was that the subjects were from the health examination population rather than the random sampling in the community, which induced sample deviation. Furthermore, our study was lack of sociological data. Previous studies had revealed that *H. pylori* infection rate was high in developing countries²⁰. Poor health conditions, low socio-economic status and associated unhealthy dietary hygiene habits might be exposed to more bacteria or antigens, which would help the immune tolerance of body to the corresponding antigens and reduce the risk of food intolerance. Therefore, the two flowers of higher *H. pylori* infection rate and lower food intolerance rate maybe both growing in the common soil of poor socio-economic conditions mentioned above. Our study had found that there might be a correlation between *H. pylori* infection and food intolerance, whether there was a causal relationship and the mechanism between them required further study.

H. pylori is considered as an important risk factor for gastric ulcer and gastric cancer. Aggressive drug therapy is recommended for patients who meet the indications²¹. However, our study found a negative correlation between *H. pylori* infection and food intolerance, which was not consistent with the criminal status of *H. pylori* as usual. If the negative correlation could be further confirmed in the future studies and the mechanism could be clarified, it would provide some advisable suggestions for medical decision.

Methods

Subjects. The physical examination data of the subjects were obtained from Health Management Centre, Sichuan Provincial People's Hospital. All the subjects completed the medical history questionnaire. Physical examination including height, body weight, blood pressure were done by the trained nurses. All the subjects accomplished laboratory examination (blood routine test, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), serum creatinine (Scr), fasting blood glucose (FBG), hemoglobin A1c (HbA1c), total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), uric acid), abdominal ultrasonography, chest imaging (X-ray or CT), ¹³C urea breath test, the food specific IgG test of egg, milk and wheat.

Subjects were excluded if (a) history of gastrectomy or subtotal gastrectomy; (b) organic diseases that have been identified to affect gastrointestinal digestion and absorption; (c) those who are unable to perform ¹³C urea breath test due to pregnancy, lactation or other reasons; (d) those who have been identified with immune system diseases, severe heart, liver and kidney dysfunction, and tumor patients; (e) history of anti-*H. pylori* therapy in the past six months.

***H. pylori* infection test.** *H. pylori* infection was determined by ¹³C urea breath tests (Beijing Boran Pharmaceutical Co., Ltd, Beijing, China), according to the recommendation of Fifth Chinese National Consensus Report on the management of *Helicobacter pylori* infection.²² Subjects have an overnight fasting for more than 8 hours, kept normal breath, inserted the straw into the bottom of one sample tube, exhaled slowly into the sample tube with the straw for 4 to 5 seconds, pulled out the straw, tighten the cap immediately, this was the sample of zero point. Then the subjects took a bottle of urea ¹³C granules with 80 to 100 ml cold drinking water, sat for 30 minutes, and then collected the breath samples again. The two collected gas samples were tested for ¹³CO₂, and used δ‰ to represent the determination result. δ‰ = (the isotopic abundance of ¹³C for test sample – the isotopic abundance of ¹³C for reference sample) × 1000/the isotopic abundance ¹³C for reference sample. The detection value was defined as the δ‰ measured in 30 minutes subtracted that in 0 minutes. *H. pylori* infection was considered positive when the detection value ≥ 4.0.

Food intolerance test. The food specific IgG screening ELISA kit (HOB Biotech Co., Ltd, Jiangsu, China) was used. Collected the serum samples of the subjects, the dosage was 5 μL, and the test was carried out according to the operation manual. Using the blank hole to adjust the zero value by the enzyme analyzer (Thermo Fisher Scientific (China) Co., Ltd, Shanghai, China) at the wavelength of 450 nm, and read the

absorbance value Y of the tested sample. According to the formula $Y = AX^3 + BX^2 + CX + D$ calculated from the standard curve, the standardized activity value X (U/ml) was obtained. Activity value $X < 50$ U/ml was defined negative (food tolerance), ≥ 50 U/ml and < 100 U/ml was defined mild intolerance, ≥ 100 U/ml and < 200 U/ml was defined moderate intolerance, ≥ 200 U/ml was defined severe intolerance.

Statement. All methods were carried out based on relevant guidelines and regulations. Ethics approval was obtained from the “Ethical Committee of Sichuan Academy of Medical Sciences and Sichuan Provincial People’s Hospital”. Informed consents were obtained from the participants or the authorized persons. Ethic approval code: No.408 (2020).

Statistical Analysis. Statistical analysis was performed using IBM SPSS 21.0 (IBM Corp., NY, USA). Continuous data were expressed as mean \pm standard deviation (SD) for normally distributed data or median with 25th and 75th percentile for non-normally distributed data. Categorical data was described in percentages. Student’s t -test was used for continuous variables. The chi-square test was used for categorical variables. Univariable and multivariable regression models were performed using the logistic regression analysis to identify the association between *H. pylori* infection and food intolerance. Various covariates such as age, sex, body mass index (BMI), HbA1c, total cholesterol, triglycerides, smoking, drinking were used for adjusting the confounding factors, with the results were expressed as odds ratio (*OR*) and 95% confidence interval (*CI*). A p value < 0.05 was considered statistically significant.

Declarations

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Ping Shuai and Ying Liu designed the study and wrote the manuscript; Dongyu Li performed the data collection and analysis; Ning Xiao and Yuping Liu assisted in data collection and manuscript writing. All authors read and approved the manuscript.

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Tables

Table 1. Demographic and Clinical Characteristics of the Participants. BMI, body mass index; SBP, systolic pressure; DBP, diastolic pressure; T2DM, type 2 diabetes mellitus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; HbA1c, hemoglobin A1c.

Variables	total (n=21,822)	male (n=12,396)	female (n=9,426)	P value
Demographic data				
Sex (female), n (%)	9,426 (43.2)			
Age (years)	43.82±10.98	44.36±10.88	43.10±11.07	<0.001
Drinking, n (%)	2,295 (10.5)	2,263 (18.3)	32 (0.3)	<0.001
Smoking, n (%)	4,578 (21.0)	4,502 (36.3)	76 (0.8)	<0.001
Anthropometric data				
Body weight (kg)	64.08±12.02	70.47±10.46	55.67±8.15	<0.001
Height (cm)	163.65±8.23	168.56±6.26	157.20±5.65	<0.001
BMI (kg/m ²)	23.81±3.37	24.77±3.18	22.55±3.19	<0.001
SBP (mmHg)	117.43±17.08	120.77±16.07	113.05±17.36	<0.001
DBP (mmHg)	72.86±11.39	75.76±11.24	69.05±10.43	<0.001
Laboratory data				
ALT (U/L)	23 (16, 36)	30 (21, 45)	17 (13, 24)	<0.001
AST (U/L)	27.20±19.03	29.71±23.13	23.90±10.75	<0.001
GGT (U/L)	24 (15, 42)	33 (22, 57)	16 (12, 23)	<0.001
Fasting glucose (mmol/L)	5.11±1.33	5.24±1.58	4.94±0.87	<0.001
HbA1c (%)	5.54±0.79	5.64±0.90	5.40±0.58	<0.001
Total cholesterol (mmol/L)	4.86±0.95	4.94±0.97	4.76±0.91	<0.001
Triglycerides (mmol/L)	1.38 (0.95, 2.08)	1.67 (1.15, 2.45)	1.09 (0.80, 1.56)	<0.001
LDL-cholesterol (mmol/L)	2.87±0.81	2.98±0.83	2.74±0.78	<0.001
HDL-cholesterol (mmol/L)	1.33±0.33	1.21±0.29	1.47±0.32	<0.001
Uric acid (μmol/L)	345.40±90.58	394.06±79.36	281.40±59.21	<0.001
Platelet count (10 ⁹ /L)	190.17±61.79	186.50±58.90	195.00±65.09	<0.001
<i>H. pylori</i> positive, n (%)	8,583 (39.3)	4,948 (39.9)	3,635 (38.6)	0.043
Food intolerance				
egg, n (%)	5,492 (25.2)	2,525 (20.4)	2,967 (31.5)	<0.001
milk, n (%)	1,973 (9.0)	982 (7.9)	991 (10.5)	<0.001

wheat, n (%)	1,031 (4.7)	445 (3.6)	586 (6.2)	<0.001
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Table 2. Prevalence of *H. pylori* infection and food intolerance in different age groups.

Age (years)	Number (n)	<i>H. pylori</i> infection [n (%)]	Food intolerance [n (%)]		
			egg	milk	wheat
18-29	1,881	607(32.3)	944(50.2)	387(20.6)	181(9.6)
30-39	6,005	2,323(38.7)	1,960(32.6)	711(11.8)	364(6.1)
40-49	7,977	3,190(40.0)	1,541(19.3)	506(6.3)	324(4.1)
50-59	3,912	1,667(42.6)	704(18.0)	242(6.2)	115(2.9)
≥60	2,047	796(38.9)	254(12.4)	84(4.1)	37(1.8)
Chi-square value		59.628	1243.420	541.111	200.009
<i>P</i> value		<0.001	<0.001	<0.001	<0.001

Table 3. The risk of the three foods intolerance according to *H. pylori* infection [*OR* (95% *CI*)] Model 1: not adjusted; Model 2: adjusted for sex, age; Model 3: adjusted for sex, age, BMI, HbA1c, total cholesterol, triglycerides, smoking, drinking.

	Model 1	Model 2	Model 3
egg	0.814 (0.764-0.867)	0.844 (0.791-0.901)	0.873 (0.812-0.938)
milk	0.714 (0.647-0.788)	0.741 (0.671-0.818)	0.751 (0.669-0.842)
wheat	0.732 (0.641-0.836)	0.762 (0.667-0.872)	0.801 (0.692-0.928)