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Seroprevalence of anti-Hp and anti-cagA antibodies among healthy persons in Golestan province, northeast of Iran (2010)

Abstract

Background: The major cause of peptic ulcer disease is helicobacter pylori (Hp), and it is also implicated in the pathogenesis of adenocarcinoma of the distal stomach and gastric lymphoma. The incidence of peptic ulcer disease, atrophic gastritis, and gastric adenocarcinoma are more common in people infected of cagA positive strains of Hp. The aim of this study was to determine the prevalence of the anti-Hp and anti-cagA antibodies among healthy persons in Golestan province-North of Iran.

Methods: The blood samples of 1028 healthy people were collected all over Golestan province by cluster sampling. A demographic questionnaire was completed and body mass index (BMI) was calculated for each case. Hp-IgG (Pishtaz teb Co. Iran) and anti- cagA (DIA.PRO Italy) titer were evaluated by Elisa method. Data were collected and analyzed.

Results: Six hundred-eighty three individuals (66.4%) were positive for Hp and 395 (57.8%) of them were cagA positive. Hp positive cases were (66.3%) and (66.6%) in male and female, respectively. Prevalence of cagA was 56.3% and 58.9%, respectively. The most seropositivity of Helicobacter Pylori (75.4%) was in 55-64 years old ($p < 0.001$). Prevalence of cagA (63.4%) was more in age between 15-24 years.

Conclusion: Prevalence of anti-Hp antibody and strains of cagA seropositive in healthy persons of this province of Iran were relatively high. Preventive protocol for reducing of the infection is recommended.

Keywords: Prevalence, Helicobacter Pylori, CagA, Antibody.

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Helicobacter Pylori (Hp) is one of the gram-negative bacteria that colonize the human stomach. The prevalence rates of this bacterium range from 25% in Western countries and over 90% in developing countries (1). The major cause of peptic ulcer disease is Helicobacter pylori, and it is also implicated in the pathogenesis of adenocarcinoma of the distal stomach and gastric lymphoma, especially the mucosa-associated lymphoid tissue-type lymphoma (MALT lymphoma) (3). The bacteria's ability to cause a disease has been associated with the expression of several virulence factors, including VacA cytotoxin, BabA, the neutrophil-activating protein (HP-NAP), outer inflammatory protein A (OipA), the duodenal ulcer promoting gene (dupA), and cag pathogenicity Island (cagPAI) (4). HP clinical isolates are classified into two types according to their pathogenicity: Type I is associated with severe disease pathology expresses functional vacA (vacuolating cytotoxin A) and contains cagA (cytotoxin-associated gene), Type II lacks cagPAI, harbors a non-toxic form of vacA and is regarded as less virulent (5). There are clusters of genes (pathogenicity island) of about 35 kilo base pairs which cagA is considered as a marker for these genes present in more than 50% of the Hp strains and encodes the 120–140 KDa (6).

cagA positive strains are more commonly associated with peptic ulcer disease, atrophic gastritis, and gastric adenocarcinoma than are cagA- negative strains (7). The aim of this study was to determine the prevalence of the anti-Hp antibody and anti-cagA antibody among the healthy subjects in Golestan province-northeast of Iran.

Methods

From 2008 to 2009, this cross-sectional seroprevalence study was carried out among the healthy people in Golestan province, northeast of Iran. Totally, 1028 persons were recruited (489 males, 539 females) aged 1-83 years. Demographic questionnaires including age, sex, residency, and ethnicity, were completed. Blood samples were stored in -70°C . After measuring weight and the height, body mass index was determined and the subjects were classified into 4 groups:

- 1- less than 18.5 kg/m^2 = thin
- 2- $24/5-25 \text{ kg/m}^2$ = normal
- 3- $29/5-25 \text{ kg/m}^2$ = .overweight
- 4- $30-39 \text{ kg/m}^2$ = .obese
- 5- More than 40 kg/m^2 = severe obesity (8).

Other variables included consuming fish, vegetables and fruits per day, past history of diabetes, smoking and blood pressure. Golestan province is classified into 3 different climates: humid mediterranean, humid semi- mediterranean and semi - dry mediterranean. We evaluated the prevalence of cagA in 3 groups.

The presence of anti- Hp antibody was evaluated by Elisa method and Hp -IgG kit (Pishtaz teb Co. Iran), and positive samples were tested for anti- cagA antibody, IgG class by anti-cagA antibody kit (DIA.PRO Italy). The data were analyzed with SPSS version 16. The prevalence of anti-Hp and anti-cagA were determined.

Results

In this study, prevalence of Hp infection was 66.4% (683/1028), which 57.7% (395/683) of them were infected to cagA. Rate of Hp-positive cases in males was (66.3%) and in females (66.6%). Prevalence of cagA in men and women were (56.3%) and (58.9%), respectively. The difference was not statistically significant. We saw minimum rates (47.7%) of Hp- cagA positive cases in the western part of the

province and maximum rates (74.4%) in the eastern part of the province.

The seroprevalence of both anti- Hp and anti- cagA antibodies increased with age, Hp infection varied from (30.6%) in children under 5 years and 71.1% above 65 years and it was most prevalent in 55-64 years (75.4%). There was significant relationship between infection of Hp and age ($p < 0.001$).

Maximum percentage of cagA positive was reported in 15-24 years (63.4%). No significant difference was seen (figure 1).

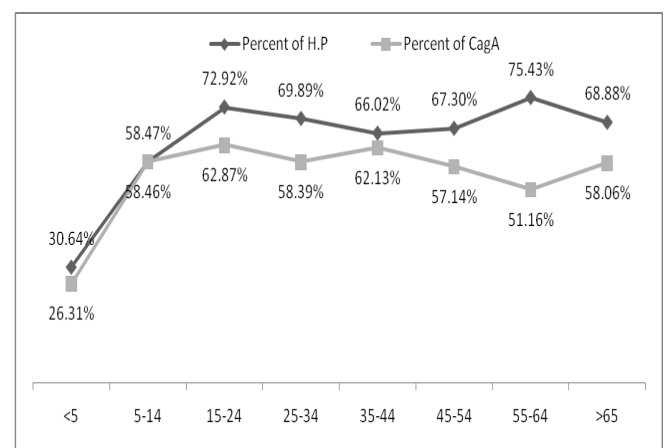


Figure1. Age distribution of Helicobacter pylori infection and cagA-seropositive in Hp⁺

There was no significant relationship between the subjects with regard to consuming fish, fruits and vegetables per day (table 1).

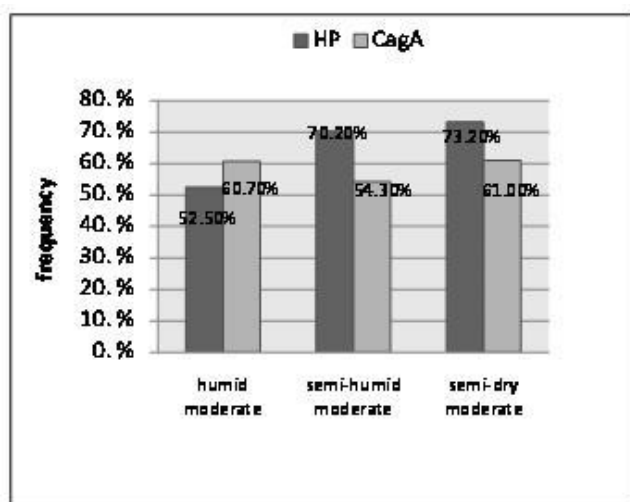
People infected with Helicobacter Pylori, were classified into three categories regarding ethnicity: Sistani, Turkman and Fars which had 70.5%, 71.2% and 61.3% prevalence, respectively ($p=0.009$). Distribution of cagA positive cases were reported as 67.2%, 57. 2%, 53.6%, in the above mentioned ethnicity, respectively ($p=0.075$).

Based on the classified climate condition, it was found that the frequency of positive Hp in humid areas was less than others (52.5%), and the frequency of cagA cases in semi- dry temperate regions are more than the other areas (61%) (figure 2).

Positive Hp cases were not different in rural and urban areas ($p>0.05$). In cagA-positive cases, rural residents showed more prevalence than urban residents (34%) but no significant differences were found ($p= 0.435$).

Table 1. Distribution of H.p and cagA seropositive regards to different variables

Factors	Positive cases of HP (%)	Positive cases of cagA* (%)
consumption of fruits and vegetables a day		
Less than once a day	69.70	55.80
1-2 portions a day	66.50	62.20
2-4 portions a day	70.30	57.30
More than 4 portions a day	66.70	40.60
Diabetes mellitus		
Yes	60.50	47.80
No	70.00	57.90
BMI		
Thin	56.60	43.90
Normal	70.70	39.30
Overweight	69.90	43.10
Obese	61.90	41.10
Morbid obesity	68.90	54.80
Smoking		
Yes	57.90	54.34
No	68.70	57.34
fish consumption in a week		
No	75.50	60.00
1 time	67.40	51.00
2 times	67.10	53.60
3times	68.30	59.30

**Figure 2. Frequency distribution Hp + (healthy individuals) and cagA+ (Hp +cases) based on the Climate**

Discussion

Hp is one of the common infectious diseases in the world (9). Approximately 20% of persons infected to Hp would progress to gastro-duodenal ulcer (10). Rising the life standards, the prevalence of Hp infection has been declined. So that contamination with this infection in developed countries were less than developing countries (11). The prevalence of this bacterium was 23.1% in Canada (12) and 32.5% in the USA (12). In developing countries, it had a higher prevalence. In several developing countries such as Albania, Kazakhstan, Vietnam and Bangladesh, the prevalence of Hp were 70%, 74%, 74.6% and 92%, respectively (14-17). In Iran, studies showed an overall prevalence of infection to be 36-71%, our study denoted that 66.8% of people living in Golestan province have IgG anti-Hp antibody and the other studies in Iran on healthy individuals have been shown the same. Prevalence of Hp was estimated 64.2% in Sari and 61.6% in Kerman (18, 19). Therefore, the pattern in our country is similar to the other developing countries. In several studies, it was shown that the prevalence of infection increased with age (17, 20, 21).

Seropositivity in studied cases in Golestan province varied from 30.6% in people under 5 to 75.4% in people aged 55-64 years. The result of the present study showed that the age trend in our region was similar to the others and infection is high in lower age. In some studies, men had more risk of Hp infection. According to the findings in Frah Naja in Canada, infection in men was 29.4% and in women was 14.9% (12). However, it was not in agreement to other studies, like Kazakhstan, Saudi Arabia and Kerman which showed no correlation with gender (16, 18, 22).

The role of nutrition in Hp infection in our study was not significant. That was consistent with the study of Khan et al. in 2007 (22). Frah Naja study showed that prevalence of infection in those who have high consumption of vegetables was less than the others (12).

In a study in Canada, no significant relationship between BMI and the prevalence of infection was seen (12). Our results showed that thin individuals (BMI less than 18.5) prevalence is lower than other groups ($p=0.02$).

In our study, no significant relationship was seen between infection and smoking. This result was consistent with Farah Naja results (12). But the study in UK by Glasgow, it showed that in smokers the prevalence of this infection was higher than non-smokers (23). The reason of high prevalence of infection in smokers is its shared use

which transfers the infection in oral- oral root (24). Hp virulence factors are important (18). In some studies in the presence of cytotoxic-associated gene in Hp strains have been attributed with gastric ulcer and cancer (25). Therefore, several researches have been done on the importance of cagA, on patients with gastric ulcer or cancer.

In this study, the prevalence of anti- cagA antibody in cases of Hp was 57.7% and it was 38.1% in the participants. High percentage of anti- cagA antibody in this region with high gastrointestinal cancers will discuss the possibility of relationship between contamination to cagA- positive Hp and cancer and also gastritis. Therefore, future researches are recommended to evaluate the relationship between gastrointestinal cancers, especially in gastric cancer cagA positive strains in this region. Age distribution of cagA antibody is similar to the distribution of Hp antibody, and was lower in the age group under 5 years-old.

One of the important findings of this study was a significant relationship between climate with existence of antibodies against cagA and Hp. Our findings showed that the probability of infection in humid climate is lower than the others. Possible causes that influence these findings are not clear to us and for confirming this authenticity and finding reasons, further studies of the climate are recommended to be able to discuss more confidently about this area.

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