Journal of Medical Bacteriology

Evaluation of Serum Levels of Anti-*Helicobacter pylori* IgG and IgA by ELISA Method in Patients with Gastric Ulcer in Chalous, Iran

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ARTICLE INFO

Article type:
Research Article

Article history:
Received: 01 Nov 2023
Revised: 28 Nov 2023
Accepted: 20 Dec 2023
Published: 02 Jan 2024

Keywords:
ELISA, Gastric Ulcer, Helicobacter pylori, IgA, IgG.

ABSTRACT

Background: Considering the role of *Helicobacter pylori* in various diseases such as chronic gastritis, gastric ulcer and malignancies, this study was performed to evaluate the level of anti-*Helicobacter pylori* antibodies in the serum of the population of Chalous and then its relationship with demographic characteristics.

Methods: A descriptive study was conducted in the department of Microbiology in a tertiary care teaching hospital during December 2019 to May 2021. All hospitalized patients with diarrhea, who had a history of exposure to antibiotic drugs and all patients with IBD were included in the study. Stool samples were collected from all patients with diarrhea who have been on antibiotic or anticancer drug during last 6weeks or previously diagnosed case. Patient's demographic features and clinical details were collected from medical records.

Results: About 78% of people had IgA positive titer and about 48% had IgG positive titer. Serum IgA level in patients with bloating (P = 0.005) and in patients with nausea (P = 0.016) was statistically significant. Also, in patients studied there was a significant relationship between bloating (p = > 0.001), nausea (0.013) was seen with a serum IgG level .

Conclusion: Due to the high percentage of positive titers of anti-*Helicobacter pylori* antibodies, especially IgA in the sample population, it seems that the follow-up of positive serological titers will help to prevent and control the prevalence of Helicobacter pylori infection.

- ***Please cite this paper as:*** Taravat A, Bashi Zadeh Fakhar H, Shaghai B, Rouhollah F. Evaluation of Serum Levels of Anti-*Helicobacter pylori* IgG and IgA by ELISA Method in Patients with Gastric Ulcer in Chalous, Iran. *J Med Bacteriol.* 2024; **12** (1): pp.9-15.

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Introduction

Infectious diseases, including bacterial infections, are among the most important diseases that have plagued human community. Thus, many efforts have been made to identify, control and treat them (1). One of the most important strategies for controlling infectious bacteria is to identify the type of pathogen and to study the prevalence of infection in an area. *Helicobacter pylori* is a type of gram-negative, spiral, curved and moving bacilli which is found pathogenic mainly in the antrum of the human stomach. This bacterium is motile thanks to the flagella (2). *H. pylori* is one of the most common infections in humans, having affected almost half of the world's population (3). This microaerophilic bacterium is in fact an opportunistic pathogen that adapts to the acidic environment of the stomach and survives this acidic environment and is the main cause of gastritis, peptic ulcers, gastric cancer and related disorders (4). *H. pyilori* was discovered by Marshall and Warren in 1983, which was one of the most important developments in medical history (5). Since then, many studies have been performed on stomach biopsy specimens from patients with acute, chronic gastritis, gastric and duodenal ulcers, and other gastrointestinal problems, and the results of these studies have proved the presence of such a pathogen in those patients. Recent studies have shown that the infection induced by this bacterium is one of the most common gastrointestinal bacterial infections worldwide, such that it has been observed in 95% of patients with duodenal ulcers and in 75% of patients with gastric ulcers. (6). The high prevalence of *H. pylori* infection in the world and its role in gastric malignancies and the emergence of antibiotic resistance has led many researchers to control this infection (7). People in developing countries may become infected with this bacterium usually in the early years of their lives, and the infection develops through digestion by ingesting the bacterium, which predisposes the person to stomach cancer by causing advanced tissue damage in the stomach. But, *H. pylori* can also be passed from person to person, as familial transmission of the infection occurs. However, the most important way of its transmission is through water (8). Acute infection can lead to the unwellness of the upper gastrointestinal tract, which is accompanied by pain and nausea, and some patients may experience vomiting and fever. Gastritis caused by this bacterium leads to complications such as gastric ulcer (lower part of the stomach or anterior region), duodenum, gastric mucosal atrophy, and gastric carcinoma. It also causes 95% of chronic gastritis, 70-80% of gastroduodenal as well as the development of gastric cancer (9).

Helicobacter pylori has been classified by the World Health Organization (WHO) as the first-line carcinogen. This pathogen is one of the most successful pathogens that infects gastric epithelial cells (27, 28). The most important step in pathogenesis of infectious microbes is the initial binding to receptors on the surface of the host cells, which binds to intracellular signaling cascade pathways, resulting in damage to the host cells and tissues.

Helicobacter pylori is initially attached to type IV collagen. This binding causes the bacteria to establish and invade the lamina propria tissue. After damaging the cell, *Helicobacter pylori* is exposed to the basement membrane and attaches to the laminin using its surface receptors, causing the bacteria to settle better in areas which lead to local injuries and wounds. Thus, the pathogensis of *Helicobacter pylori* can play a role in causing cancer (29, 30).

The prevalence of *H. pylori* infection varies widely in developed and developing countries, ranging from 25% in developed countries to more than 90% in developing countries. It is highly prevalent in Eastern Europe, Asia, Africa, and South America (10). Numerous studies on healthy individuals in Iran have shown that about 70 to 80% of adults have *H. pylori* infection, which is less than Portugal with the highest rate of prevalence (84%) (11).

ELISA is one of the main diagnostic methods to detect *H. pyilori* (12).

Considering the importance of *H. pylori* infection, this study was performed in which ELISA method evaluated the level of anti-*H. pylori* IgA and IgG antibodies in the serum of patients with gastric ulcer. In the present study, in addition to examining and comparing the titers of IgA and IgG antibodies in the serum of patients, other factors such as relationship between age, sex, history of bloating, nausea, family gastric cancer, history of *H. pylori* and previous treatment records of patients with specific antibody titers against the target bacterium were compared.

Materials and Methods

This descriptive study was performed on 50 patients with gastric ulcer referred to Chalous Medical Diagnosis Laboratory during June 2020 to August 2020. Inclusion criteria included the patients greater than or equal to18 years of age and those who were referred for endoscopy according to the aforementioned symptoms. Exclusion criteria included use of any antibiotics, bismuth, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids or proton pump inhibitors (PPIs) during the previous 2 weeks, recent upper gastrointestinal (GI) bleeding, pregnancy, acute or chronic renal failure, chronic liver disease, age less than 18 years, gastric carcinoma and diabetes mellitus. Before collecting the samples, a questionnaire containing several personal questions, including age, sex, history of gastrointestinal disease, history of use of certain drugs affecting the gastrointestinal tract, etc., was given to the patient and all personal questions related to the patient's file was given to the center with observing confidentiality. After completing the questionnaire and consent forms by the patients, sampling was performed by receiving five cc of intravenous blood.

The samples were clustered randomly selected from each age group and the titers of serum IgA and IgG antibodies against *H. pylori* were measured by indirect ELISA method using ELISA kit of Pishtaz Teb company. The lgG and IgM titer was reported negative for values lower than 10 and positive for titers of 10 and higher. Calculation of mean data and comparison of antibody titers with other parameters were analyzed using SPSS software as well as t-test and ANOVA. During data comparison, p value less than 0.05 is statistically significant.

Result

In this descriptive study, 50 individuals with gastric ulcers with an average age of 41.5 ± 15.2 referred to Chalous Medical Diagnosis Laboratory in June and August 2020 were studied. The majority of these people (48%) were over 40 years old. The percentage of men (56%) was about 12% higher than women (44%). The majority (58%) had clinical signs of bloating. Also, 10% of patients had a history of bacterial infection and only one person (2%) had a history of gastric cancer in the family. About 18% of people had a history of previous treatment and 12% had clinical symptoms of nausea. Some demographic information about patients is summarized in Table 1 and the mean antibody titers along with other parameters are presented in different groups.

Table 1. Distribution of disease-dependent individual variables and IgG and IgA antibodies in the subjects.

Variable		No.	Percent
Sex	Female	28	56%
	Male	22	44%
Age group	Under 30	13	26%
	31- 40	13	26%
	Above 40	24	48

Age	Standard deviation ± mean (minimum – maximum)	41.5 ± 15.2 (14 - 80)	
Previous treatment records	Yes	9	18%
	No	41	82%
Abdominal bloating	Yes	29	58%
	No	21	42%
History of Helicobacter pylori	Yes	10	20%
	No	40	80%
Gastric cancer in the family	Yes	1	2%
	No	49	98%
Gastric ulcer	Yes	50	100%
	No	0	0%
Nausea	Yes	6	5 12
	No	44	88%
G_IgA	N	11	22%
	P	39	78%
	Standard deviation ± mean (minimum - maximum	30.2 ± 18.1 (8.20 – 82.20)	
G_IgG	N	26	5 52
	P	24	48%
	Standard deviation ± mean (minimum - maximum	32.6 ± 36.3 (1.19 – 179.40)	

Serum IgA level in patients with bloating was (P = 0.005) and in patients with nausea (P = 0.016) which are statistically significant. These patients also had a higher mean and median of serum IgA levels. Mean and median of serum IgG levels in the age group under 40 and above were lower than other age groups (P 0.002). Also, in this study on sample patients, there was a significant relationship between bloating (p = > 0.001), nausea (P = 0.013) was seen with serum IgG levels, which had a higher mean and median than other variables, but no significant difference was seen in other clinical signs.

Only one person (2%) had a family history of gastric cancer. Also, 12% of the samples had clinical symptoms of nausea. Helicobacter IgA and IgG were positive in 78% and 48% of patients, respectively. The mean and standard deviation were 30.2 ± 18.1 and 32.6 ±36.3, respectively.

About 78% of the participants in this study had a positive IgA titer and about 48% of the subjects had a positive IgG titer. In terms of gender, 82.1% of women and 72.7% of men had IgA positive titer and 46.4% of women and 50% of men had IgG positive titer.

Discussion

One of the most common infections in humans is *H. pylori*, which is transmitted orally and through fecal–oral route. It is claimed that this bacterium has infected about half of the world's population (13). The results of researches conducted in recent years in different parts of Iran have reported the prevalence of this infection between 34% to 61% (14). Various studies have shown an association between the presence of *H. pylori* and various diseases of the gastrointestinal tract, including chronic gastritis, gastric ulcer, and gastric adenocarcinoma. A remarkable result of some research is that people with high IgG levels are more likely to develop stomach cancer (15).

Serological tests are useful because of their low cost, availability, and high sensitivity for the early diagnosis of infection (12). The results of serological tests are very valuable in patients with peptic ulcer bleeding who have negative urease test and histology results, as well as in cases of active acute gastritis. (16).

When *Helicobacter pylori* penetrates into the tissues and colonizes the gastric mucosa, the level of IgA class antibodies increases. Even, at this stage of the disease, clinical symptoms may not be observed for a long time (17). According to studies, in patients with recurrence of infection with this bacterium, the level of IgA antibody titer increases before the serum IgG antibody titer. If *Helicobacter pylori* spreads to other organs and causes infection with clinical symptoms, first the seram level of IgM class antibodies and after a while, IgG class antibodies are increased, which indicates that the disease is chronic (18).

The results of this study showed that about 78% of the participants in this study had a positive IgA titer and about 48% had a positive IgG titer. In terms of gender, 82.1% of women and 72.7% of men had IgA positive titer and 46.4% of women and 50% of men had IgG positive titer. Serum IgA level in patients with abdominal bloating (P = 0.005) and in patients with nausea (P = 0.016) was statistically significant. These patients also had higher mean and median of serum IgA levels. Mean and median of serum IgG levels in the age group under 40 and above were lower than other age groups (P 0.002). Additionally, in patients under study, there was a significant relationship between abdominal bloating (p = > 0.001) and nausea (P = 0.013) was seen with serum IgG levels that had higher mean and median than other variables, but no significant difference was seen in other clinical signs.

Various studies have been performed on the prevalence of *H. pylori* infection in serum antibody titers, which are comparable to our research. In a cross-sectional study, Sadeghi et al performed a study on 331 individuals over 11 years of age in health centers in Kerman and measured the rate of serum IgG antibody titer against *H. pylori*. The results of their study showed that the total relative frequency of serologically positive infection against *H. pylori* was 61.6%. There was no significant difference between men and women in terms of infection with this bacterium. The rate of infection in the younger age groups of the population was almost similar to adults and the elderly and no significant difference was observed based on the age of individuals with the level of *H. pylori*. In addition, the rate of infection with lower education showed a significant increase (19).

In a study conducted by Baba Mahmoudi et al. (2004) on 394 residents of Sari city and villages around it, they showed that 64.2% of the subjects were infected with *Helicobacter pylori* and by aging, the rate of infection would significantly increase. The results of their data analysis showed that the age variable was most associated with the prevalence of infection (20).

In another study, Chupani et al performed serum IgG antibody titer against *H. pylori* on 516 patients referred to the central laboratory in the south of Tehran. The results of their study showed that out of the total number of patients, 156 people (30.2%) were IgG positive, of which 51 were male (32.7%) and 105 (67.3%) were female. The percentage of positive cases among men (43.5%) alone was higher than women (26.5%) and women over 45 years had the highest percentage of positive cases (21).

In another study conducted by Mina Orang et al. (2014) on 400 men and women referred to Sari medical centers, no significant difference was observed between men and women in terms of positive or negative IgA antibody titers. About 18.5% of men and women had positive IgA titers and about 70.2% of men and 66.7% of women had positive IgG titers. The mean antibody titers in people with a history of gastrointestinal infections was significantly higher than other people (22).

In another study, Gholizadeh et al. (2014) measured serum IgG antibody titer against *H. pylori* in 272 students of Gachsaran Islamic Azad University. The mean amount of antibody against *Helicobacter pylori* infection was 32.75 units per ml. 67.6% of the results were positive, 16.8% were

negative and 15.6% were in the border range. The results of their study didn't show a significant difference in the serological prevalence of *Helicobacter pylori* infection according to age, sex, blood type, number of family members, family income, smoking and alcohol consumption, history of gastrointestinal symptoms in the subjects or their family members and hygienic habits such as washing hands before food and after defecation (23).

In another study, Sadeghi et al. (2020) performed serum IgA, IgG and IgM antibody titers against *H. pylori* in 650 patients referred to Islamshahr Health Center. Of the 650 samples tested, 47.08% were IgG, 14.62% IgA and only 4.62% IgM positive. The highest frequency of IgG antibodies was observed in the age group of 46-60 years. There was no significant relationship between the mean titers of IgA, IgG and IgM antibodies with age (24).

In another study performed on 100 patients with gastritis and duodenitis referred to Imam Khomeini Hospital in Ahvaz, serum IgG and IgA antibody titers against *H. pylori* were measured. Of the 650 samples tested, 82% were IgG and 39% were IgA positive. As for the control group, 53 people with no history of gastrointestinal disturbances and of the same age and sex as patients were selected and the said antibodies were measured in them. The results showed that the abundance of both classes of antibodies in patients was significantly different from that of the control group, and gender did not play an important role in antibody abundance (22).

According to the results of this study and other related studies, *H. pylori* infection has been observed at any age and sex, which generally has no specific symptoms in the early stages of infection. Since our country is considered a developing country and a large percentage of the population may be in the lower class in terms of quality of life, health and level of education, tracking the titers of these antibodies in these people is very important. It is valuable since it helps a lot to manage the control and prevention of the spread of infection caused by this bacterium. Besides, the number of gastrointestinal infections in our country has a high percentage, which is likely to be associated with the prevalence of these infections with the said bacterium and requires necessary measures to be taken subsequently (25).

Conclusion

Our results suggest that the cut-off value in the serological technique should be increased according to the patient's age. Moreover, there is a clear relationship between the serum levels of antibody's and the activity of gastritis. It is clear that more research is needed. Further studies should also be performed on a larger community of Patients with gastric ulcer in this geographical area.

Acknowledgements

We sincerely thank the full cooperation of experts and officials of health centers and medical diagnostic laboratories of Chalous city who cooperated in different stages of this research.

Funding Information

This research received specific grant from faculty of veterinary medicine, University of Tehran.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki and its later amendments or comparable ethical standards. Also ethics committee of Azad University of Medical Sciences, Iran approved this study by (Code no: IR.IAU.CHALUS.REC. 1399.016).

Conflict of interest

The authors declare that they have no conflict of interest.

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