Comparison of Serum IgG and IgA Levels against *Helicobacter Pylori* in Patients with Gastrointestinal Symptoms

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ABSTRACT

Aims *Helicobacter pylori* is a pathogen that can be colonized in the stomach. Most laboratories only use IgG and not IgA antibody to diagnose infection. The aim of this study was to compare both IgG and IgA-antibodies level for the detection *H. pylori*.

Materials & Methods The presence of IgG and IgA antibodies in the sera of the 517 patients suspected to *H. pylori* infection was evaluated by Enzyme-Linked Immunoadsordent Assays (ELISA) method.

Findings The positive cases of infection on the basis of IgG and IgA titers were 68% and 27%, respectively. Also, 7% of the patients with IgG negative were IgA positive.

Conclusion The comparison of antibody responses in our patients indicate that the sensitivity of IgA level is lower than IgG ELISA and both antibody titers must be evaluated for the identification of infection. In some cases, patients with IgG negative may have IgA positive assays; therefore, in the serological diagnostic process and without endoscopy, IgG results in association with IgA against *H. pylori* will be completed.

Keywords *Helicobacter pylori*;ELISA; IgG; IgA

CITATION LINKS

[1] Antimicrobial activity of Elettaria cardamomum: Toxicity, biochemical and histological studies [2] Campylobacter [3] Gastric cancer [4] Management of Helicobacter pylori-related disorders [5] IL-10, TNF-α and IFN-γ levels in serum and stomach mucosa of Helicobacter pylori-infected patients [6] Impact of Helicobacter pylori immunoglobulin G levels and atrophic gastritis status on risk of metabolic syndrome [7] Effect of Helicobacter pylori eradication on elder cases: Observational study in community-based medicine [8] Gastric mucosal immunity induced by H. pylori infection [9] Diagnostic value of decreasing IgG, IgA, and IgM antibody titres after eradication of Helicobacter pylori [10] Mucosal production of antigastric autoantibodies in Helicobacter pylori gastritis [11] Immune responses to Helicobacter pylori infection in Bangladeshi children during their first two years of life and the association between maternal antibodies and onset of infection [12] Detection of anti-Helicobacter pylori antibodies in serum and duodenal fluid in peptic gastroduodenal disease [13] Evaluation of serology as a diagnostic method for Helicobacter pylori infection in the local population of Guayaquil [14] Diagnostic value of detection of IgM antibodies to Helicobacter pylori [15] Anti Helicobacter pylori IgG and IgA response in patients with gastric cancer and chronic gastritis [16] Non-invasive diagnosis of Helicobacter pylori: Evaluation of two enzyme immunoassays, testing serum IgG and IgA response in the Anand district of central Gujarat, India [17] Validation of diagnostic tests for Helicobacter pylori with regard to grade of atrophic gastritis and/or intestinal metaplasia [18] Immunoglobulin G antibody against Helicobacter pylori is an accurate test for atrophic gastritis [19] Detection of Helicobacter pylori by real-time PCR for 16s rRNA in stools of noninfected healthy children, using ELISA antigen stool test as the gold standard [20] Evaluation of Helicobacter pylori Immunoglobulin G (IgG), IgA, and IgM serologic testing compared to stool antigen testing [21] Evaluation of two commercial enzyme immunoassays, testing immunoglobulin G (IgG) and IgA responses, for diagnosis of Helicobacter pylori infection in children [22] Comparison of serum IgA and IgG antibodies for detecting Helicobacter pylori infection [23] Diagnosis of Helicobacter pylori infection by using pyloriset EIA-G and EIA-A for detection of serum immunoglobulin G (IgG) and IgA antibodies [24] Circulating anti-Helicobacter pylori immunoglobulin A antibodies and low serum pepsinogen I level are associated with increased risk of gastric cancer [25] Serum Helicobacter pylori IgG and IgA levels in patients with gastric cancer [26] Immunoglobulin A antibodies to Helicobacter pylori [27] Serum anti-Helicobacter pylori IgA and IgG antibodies in asymptomatic children in Serbia [28] IgG and IgA antibodies in Helicobacter pylori infections

Introduction

Helicobacter pylori is a helical, gram-negative, and motile bacterium that is the causative agent of active chronic gastritis and stomach and duodenal ulcers and non-ulcer dyspepsia. Infection with virulence strains increases the risk of gastric mucosa-associated lymphoid tissue [1] lymphoma and gastric cancer.

The World Health Organization (WHO) [2] recognized organism as a group I carcinogen for human in 1994 [3-5]. Some of its extra-gastrointestinal symptoms are reported, including anemia, obesity, vitamin deficiency, diabetes mellitus, cardiovascular diseases, and hyperlipidemia, etc. [6-7].

In infected individuals, both cellular and humoral immune responses were elicited [8]. In the early stages of the disease, there are ratio high IgM antibodies, while in the later stages of the disease, there is a ratio high of IgG antibodies in serum. IgG response can also be observed for a long period in the human body unless the pathogens are eradicated completely [9]. In addition, secretory IgA antibody responses are stimulated against the bacteria in both gastric juice and serum [10-12].

Today, one of the non-invasion techniques in disease diagnosis is serology tests that are commercially available and easily performed in most laboratories with low cost [13].

The levels of serum anti-*H. pylori* antibodies in the different stages of the disease are important. The studies showed that increased IgM titer is as an indicator of acute disease, but increased IgG/IgA levels are as an indicator during chronic infections [14, 15]. Most medical laboratories only use IgG-based tests and an IgA assay in serum is less well documented.

Therefore, the aim of this study was to evaluate IgG and IgA antibodies serum levels in patients with gastrointestinal symptoms.

Materials and Methods

In this study, 517 patients with gastrointestinal symptoms and suspected to *Helicobacter pylori* infection were admitted to the Shahid Beheshti Hospital, Qom, Iran during a 5-month period. These patients had undergone endoscopic procedures in the last month and had a positive urease test. Due to digestive problems, including chronic gastritis, gastro-oesophageal reflux disease, abdominal pain or other symptoms compatible with *H. pylori* infection, were returned to a specialist of gastroenterology. The satisfaction of the patients was obtained by oral explaining and, then, the informed consent form was studied and signed by them.

Overall, inclusion criteria were a history of positive test for *H. pylori* and a conscientious consent to participate in this study. 10 ml blood sample was obtained from each patient and, then, the serum was separated by centrifugation in 2000×g for 15 minutes. The serum samples for the measurement of antibodies were frozen in -20°C.

The presence of anti-*Helicobacter pylori* immunoglobulins in serum of patients was evaluated, using the Enzyme-Linked Immunoadsordent Assays (ELISA) method according to kit instructions (Padtan Elm Co., Ltd; Iran). All experiments were performed in duplicate. The IgA/IgG ELISA titers >20U/ml were considered as positive for *H. pylori*. Finally, antibody levels of patients were presented as the mean ± standard deviation (mean ± SD).

Findings

31% and 69% of the patients were male and female, respectively. Totally, 95% of the patients were positive for *H. pylori* with a titer >20U/ml, while 5% of them were negative and showed less than the standard amount. Among individuals, the IgG and IgA antibodies against *H. pylori* were detected in 353 (68%) and 138 cases (27%), respectively. Testing for 7% of the patients (37 cases) with IgG negative were IgA positive. In addition, 19% of the patients were positive for both antibodies (IgG and IgA). According to endoscopic results as a gold standard, the sensitivity was 68% for IgG and 26% for IgA tests. The specificity of the serology assays was 100%. Among male and female, mean ± SD of the serum concentrations of IgG and IgA were 61±22.75 U/ml and 37±17.68 U/ml, respectively. The rate of two antibodies was higher in females than in males. In addition, there was no relationship between clinical symptoms and antibody levels. The mean ± SD of positive cases on the basis of gender are presented in Table 1.

Table1) The mean ± SD of positive cases on the basis of gender

Variable	Male	Female
IgG (U/ml)	60±23.60	61±22.44
IgA (U/ml)	36.5±17.49	37±17.85

Discussion

The importance of *H. pylori* infection in gastrointestinal tract is well recognized worldwide, especially in Iranian populations. Both local and systemic antibody responses against *H.pylori* infection can be stimulated.

At the beginning of the infection, the first immunoglobulin in a systemic response is IgM, followed by a rise in specific IgA and IgG levels. Usually, *H. pylori*-positive people have raised levels of specific IgG antibodies, but IgA titer may be increased only about 70% of the patients [16].

Today, immunological tests are widely available in diagnosis of diseases and epidemiological studies. In the different studies, it was determined that the

sensitivity and specificity of serological tests is highly variable [17, 18], but most clinical laboratories routinely are using antibody-based assays for the detection of the microorganisms.

The use of these methods for evaluating *H. pylori* in stool specimens has shown sensitivity of ~85% and specificity of ~95%, compared to other invasive methods [19].

However, they also are mostly useful for screening of *H. pylori*-infected patients before endoscopy for the presence or absence of the bacteria and effective management of treatment among infected patients.

Due to some general clinicians ordered only IgG and/or IgA tests for the identification of the *H. pylori* and without endoscopy, we would like to know whether the evaluation of antibodies separately, with no additional procedures, could be a good way to predict the disease in patients with a history of endoscopy. So, the study was done on some patients with a history of endoscopy and a positive urease test. In our study, 95% of the patients were positive for *H. pylori* and the results were related to endoscopic findings. 5% also showed a titer less than 20U/ml and were considered as a false negative result. The serum concentration of IgG in patients was remarkably higher than IgA. This finding showed that the sensitive of IgA-ELISA is lower than IgG-ELISA (68% vs. 26%). The data are in agreement with a study conducted by She *et al.*, who determined a sensitivity of IgA, IgG, and IgM antibodies against *H. pylori* stool antigen to 63.4%, 87.6%, and 6.8%, respectively [20].

In another study conducted by Kindermann *et al.*, IgG and IgA responses for the diagnosis of *H. pylori* infection in children were evaluated. They showed that the sensitivity test for IgA-based assays was less than IgG-based assays [21].

In addition, Pandya *et al.* showed that IgG ELISA is a reliable and accurate test for the evaluation of *H. pylori* and it can be useful as a screening assay and an alternative method to endoscopy [16].

According to the obtained results, although the sensitivity of IgA titer was low, it seems that its specificity was higher. This result is consistent with studies conducted by Urita *et al.* and Granberg *et al.*. Due to high specificity, they proposed that IgA antibody test should be performed in clinical laboratories for the detection of *H. pylori* [22, 23].

In addition, Granberg also determined that the presence of IgG and IgA is correlated with active infections in 95% (duodenal ulcer) and 74% (gastric ulcer) [23].

In another research, Aromaa *et al.* indicated that the presence of anti-*H. pylori* IgA, and serum pepsinogen I is related to increased risk of stomach cancer [24].

It was suggested in another study that IgA test can be useful as a better predictor of *H. pylori* infection in patients with gastric cancer [25].

Our data also show that the frequency of the patients with IgA positive alone was 7%. This percent is similar to the results of studies performed by Jaskowski *et al.* and Dinić *et al.* with a range from 4.9% to 7.2%. Jaskowski *et al.* also suggested that an IgA-positive result of the patient to *H. pylori* with intestinal symptoms may be an important clinical value for the detection of the disease, especially if IgG test is negative [26, 27]. Therefore, it seems that a few *H. pylori*-infected patients with IgG negative may be missed in the diagnostic process when evaluated alone.

Gościniak *et al.* also showed that the consideration of IgA antibody in human samples may be used as an assay that complements IgG antibody assay [28].

Furthermore, in this study, although antibody levels in female was a little higher than male, significant difference was not observed between both male and female.

Due to financial limitations, we were unable to evaluate IgM ELISA and gene expression of the antibodies by molecular assays. Also, the correlation between antibody levels and virulence factors was not studied in this work.

Conclusion
This report shows that in the detection process of the disease by serological tests and without endoscopy, IgG ELISA results in association with IgA results will be concluded.

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