Anti-CagA IgG Antibody Is Independent from Helicobacter pylori VacA and CagA Genotypes

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ABSTRACT

Background: Helicobacter pylori strains have two classical virulence genes, the cytotoxin-associated A (cagA) gene and the vacuolating cytotoxin A (vacA) gene, which are located in the cag pathogenicity island (cagPAI). Serum immunoglobulin G (IgG) antibodies to H. pylori, especially, the CagA antigen may be a reliable marker for selection of dyspeptic patients for upper endoscopy.

Methods: Serum sample of 129 dyspeptic patients with positive H. pylori, were tested for serum IgG Anti-CagA antibody by ELISA. The presence of the cagA and vacA genotypes were determined using polymerase chain reaction (PCR) on biopsy samples taken via endoscopy.

Results: Positive serum IgG anti-CagA antibodies in patients with cagA+/vacA+ and cagA+/vacA− genotypes were 22/23 (95.6%) and 18/19 (94.7%), respectively. In addition, serum IgG anti-CagA antibodies in patients with cagA+/vacA+ and cagA+/vacA− genotypes were 22/47 (46.8%) and 33/40 (82.5%), respectively.

Conclusion: It can be concluded that the serum IgG anti-CagA antibody alone could select patients with dyspepsia following upper endoscopy. The assessment of vacuolating cytotoxin activity of H. Pylori is, therefore, not required, even when vacA gene is positive. This hypothesis needs to be studied in a large number of patients with dyspepsia.

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Introduction

*Helicobacter pylori* is a gram-negative, microaerophilic bacterium that colonizes more than half of the world’s human population. Despite the development of gastric mucosal inflammatory response and *H. pylori*-specific humoral immune response, *H. pylori* can persistently colonize the stomach for decades or for life. Most individuals harboring *H. pylori* remain asymptomatic, but the presence of this organism is a risk factor for the development of peptic ulceration, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma (1-3).

The *cag* pathogenicity island (*cag*PAI) is the most virulence factors of *H. pylori* which are frequently associated with the most serious clinical outcomes. The *cag*PAI can be divided into two parts: the upstream *cagII* and the downstream *cagI* regions by an insertion sequence called IS605. The *cagA* gene is located in the most downstream portion of *cagI* and is known as a marker for the *cagI* region. The *cagA*-positive strains are reported to be related to severe clinical outcomes, especially in Western countries (4). Two virulence factors have been found more frequently in *H. pylori* strains isolated from patients with ulcers or cancer than in strains isolated from patients with gastritis. The former is the cytotoxin-associated antigen (*CagA*) which reflects the presence of the *cag* PAI, including about 30 genes of unknown function (5, 6). Expression of *CagA* protein is closely associated with that of vacuolating cytotoxin, although the underlying mechanism is not understood. Another important virulence factor of *H. pylori* is a vacuolating cytotoxin activity (*VacA*), which is associated with injury of epithelial cells. The *vacA* gene present in nearly all strains of *H. pylori*, but it is polymorphic, comprising variable signal regions (type s1 or s2), mid-regions (type m1 or m2) and recently i region (7, 8). Furthermore, vacuolating cytotoxin activity expression blocks T-cell activation, proliferation and inhibits antigen presentation in T cells. In addition, antigen processing by B lymphocytes is blocked, possibly by limiting the maturation of endosomes to MHC class II compartments where antigen loading takes place. Therefore, vacuolating cytotoxin activity limits T and B-cell response against *H. pylori* (9-11).

Chronic *H. pylori* infection elicits local and systemic immunologic response leading to production of IgG antibody. IgG antibody can be detect against whole-cell preparation in serum and also against cytotoxin-associated protein. This protein is highly immunogenic and usually stimulates immune response and can be measured by ELISA and western blotting system (12).

It has been shown that a specific antibody response pattern is one of the most reliable methods for diagnosis of *H. pylori* infection especially peptic ulcer disease. The aim of this study was to extend the data and to search for specific antibody patterns in sera from *H. pylori* patients having *cagA* gene with or without *vacA* gene, in patient with dyspepsia.

Materials and Methods

Patient Samples

The present study carried out in 130 patients with the chief complaint of dyspepsia with over 2 month duration who were referred to the endoscopy ward of Firoozgar Hospital. The patients, who were aged between 16 and 69 years, were selected according to Leeds Medical School criteria (13). All the patients provided informed consent and accepted to complete a standard questionnaire. Esophago-gastroduodenoscopy (EGD) was done for all the patients in the same center by expert endoscopists. The patients with dyspepsia with or without peptic ulcers were selected and included in the study. The eligibility of the patients was based on the results of the questionnaire and EGD. Those patients who had no history of proved ulcer such as previous *H. pylori* eradication, cigarette smoking, malignancy and other underlying diseases in EGD, and did not use antibiotics (at least 2 months before endoscopy) were excluded from the study. Four biopsy specimens were taken from the antrum and...
gastric body for histological study and *H. pylori* detection. All the formalin embedded specimens were fixed and stained with Hematoxylin and Eosin Stain (H&E) or Geimsa. The specimens were evaluated by an experienced pathologist. A patient was considered to be positive for *H. Pylori* when at least 5 bacilli in each microscopic field were found. Five ml of the blood sample was taken and sent to the Immunology laboratory for anti-CagA antibody measurement. Antibody measurement was performed using the ELISA commercially supplied (Diagnostic Bioprobes- Italy). The anti-CagA antibody in the patients’ sera was based on the synthetic CagA coated microplates. The complex was detected by HRP conjugated antisera, and the color that developed was measured in 450-nm filters prefiltred at 620 nm. The optical density (OD) of each well was measured and the antibody level was calculated using a standard calibration curve. The method is both sensitive and specific (98%), and its diagnostic sensitivity and specificity are more than 98%. A value ≥ 5 Arbitrary/ml was considered as positive for the anti-CagA antibody.

**PCR**

The genomic DNA was extracted from the biopsy samples using a DNA isolation kit for cells and tissues (Roche Applied Science Company, Germany) in accordance to the manufacturer's instruction and stored at -20 °C. Two sets of primers were designed complementary to the sequence located within the conserved region of the gene primers; cagA1 and cagA2. The primers sequences and size are depicted in Table 1 (14). The amplified products were detected after agarose gel electrophoresis with a 340 bp which indicated the presence of the *H. pylori*-cagA in the specimen (Figure. 1). The identification gene product belong to *H. pylori* was confirmed via the PCR for the GlmM (urea C), a conserved gene, specific for the bacteria (14). The identification of vacA gene was confirmed by PCR using primers specific for its signal sequence with the pair of primers; vacA1 and vacA2. The characteristic of the primers is illustrated in table1. The amplified product was evident as above with 480 bp band which indicates the presence of the *H. pylori* vacA in the specimen (Figure. 2).

**Statistical Analysis**

All the data were analyzed using SPSS software (version18) for each patient. Age is shown with ± standard deviation. The effects of vacA gene positive on the serological response were expressed as odds ratios (ORs) with 95% confidence intervals (CIs) with reference of vacA gene negative patients having *H. pylori* infections. The results were compared by Mantel-Haenszel chi-squared test with Yates correction or Fisher exact probability test. A P-value less than 0.05 was considered statistically significant.

**Results**

Overall, 130 patients, with ulcer and non-ulcer dyspepsia were selected based on the results of a questionnaire and esophagogastroduodenoscopy. One of these patients had vacA gene positive and negative result which was excluded. The remaining of 129 patients, 42/129 (32.5%) and 87/129 (67.5%) had cagA gene positive and negative, respectively. The average age was 42.9 ±12.1 (16 to 64) years in the cagA gene positive

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**Table 1. Characteristics of the primers used for the detection of cagA and vacA genes.**

<table>
<thead>
<tr>
<th>Amplified region</th>
<th>Primer designation</th>
<th>Primer sequence</th>
<th>Product size (bp)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>cagA1</td>
<td>cagA1-F</td>
<td>5’-AAG ACC AAT ACG GGT TAT TTG-3’</td>
<td>348</td>
<td>14</td>
</tr>
<tr>
<td>cagA2</td>
<td>cagA2-F</td>
<td>5’-TG TAA CAC AAG ACA AGG AAG-3’</td>
<td>383</td>
<td>15</td>
</tr>
<tr>
<td>vacA1</td>
<td>vacA1-F</td>
<td>5’-ATG GGA ATT CAG CAG CGA-3’</td>
<td>480</td>
<td>14</td>
</tr>
<tr>
<td>vacA2</td>
<td>vacA2-F</td>
<td>5’-AGG AAG ACT TGG CAG AGA G-3’</td>
<td>480</td>
<td>14</td>
</tr>
</tbody>
</table>

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Overall, 130 patients, with ulcer and non-ulcer dyspepsia were selected based on the results of a questionnaire and esophagogastroduodenoscopy. One of these patients had vacA gene positive and negative result which was excluded. The remaining of 129 patients, 42/129 (32.5%) and 87/129 (67.5%) had cagA gene positive and negative, respectively. The average age was 42.9 ±12.1 (16 to 64) years in the cagA gene positive
patients and 40.9±14.7 (16-64) years in patients with cagA gene negative. Amongst the patients, 20/42 (47.6%) and 45/87 (51.7%) of patients were female in cagA gene positive and negative patients, respectively. Also, 23/129 (17.8%) and 19/129 (14%) of patients had cagA+/vacA+ and cagA+/vacA−, respectively. In addition, 12/23 (52%) of patients with cagA+/vacA+ and 7/19 (36.8%) of patients with cagA+/vacA− were females. The mean age ±SD of patients with cagA+/vacA+ was 39.0±13.6 (16-67) years and the mean age ±SD of patients with cagA+/vacA− was 40.0±14.7 (18-69) years.

The patients with cagA gene positive group, 23/42 (54.8%) and 19/42(45.2%) had vacA gene positive and negative, respectively. The patients with cagA gene negative group, 47/87 (54%) and 40/87 (46%) had vacA gene positive and negative, respectively. Serum IgG anti-CagA antibodies positive in patient with cagA+/vacA+ and cagA+/vacA− genotypes were 22/23 (95.6%) and 18/19 (94.7%), respectively. Serum IgG anti-CagA antibodies in patients with cagA−/vacA+ and cagA−/vacA− genotypes were 22/47 (46.8%) and 33/40 (82.5%), respectively. These results are presented in table 2. The relation of serum IgG anti-CagA antibodies between two groups, cagA+/vacA+ and cagA+/vacA− were not significant (odds ratio: 1.2, 95% CI: 0.8-16.6; p=0.9) (Table 3).
Discussion

Our results did not show the relation of IgG anti-CagA antibodies in patients with cagA+/vacA+ genotypes (22/23, 95.6%) and cagA+/vacA− (18/19, 94.7%) genotypes in patient with dyspepsia (odds ratio: 1.2, 95%CI: 0.8-16.6; p=0.9). The serological response to H. pylori antigens were heterogeneous. Although, the serum IgG anti-CagA antibody results might be different in another study with H. pylori-vacA alleles, suggesting that some patients with vacA gene positive do not have vacuolating cytotoxin activity.

The vacA gene of H. pylori encodes for VacA, a secreted vacuolating cytotoxin, which induces a vacuolating cytotoxic effect in gastric cell lines. The vacA is a polymorphic gene and both active and inactive forms of the toxin exist. Strains of H. pylori that express active forms of the toxin are associated with more severe cases of the disease. Three regions of variation have been defined and there are at least two primary variants in each region; the regions are designated as the signal (s), intermediate (i), and middle (m) regions. Furthermore, strains carrying vacA s1, i1, m1, and combinations of these alleles are overall associated with more severe diseases. Each region was found in different location of the genome and have distinct function (8). A large variety of additional cytotoxic functions has been attributed to VacA in the last 10 years of extensive characterization, such as altering the endosomal function, inhibiting T-cell proliferation, internalizing and damaging mitochondria, and inducing apoptosis (15).

The cagPAI is a pathogenicity island approximately 40 kb in size containing about 30 genes including those which encode the type IV secretion system (TFSS), a syringe-like structure responsible for transfer of the CagA protein from H. pylori into the host cell (5, 6). Xiang et al classified H. pylori strains into two groups, type I and type II. Type I strains is positive for both cagA gene and vacuolating cytotoxin activity and type II strains positive for cagA gene without vacuolating cytotoxin activity, although vacA gene is present. The gene coding for the vaculating cytotoxin, VacA, is polymorphic and present in all strains, but various strains have shown marked differences in the production of vaculating cytotoxins. Type I was strongly associated with peptic ulcer diseases in the host (7). When the cagA positive gene are presented, immune system usually have been exposed to the CagA antigen, especially since CagA is injected into epithelial cell of stomach by TFSS which is encoded by other genes contained within the PAI. The vacuolating cytotoxin activity expression blocks T-cell activation, proliferation and inhibits antigen presentation in T cells. In addition, antigen processing by B lymphocytes is blocked, possibly by limiting the maturation of endosomes to MHC class II compartments where antigen loading takes place. Therefore, vacuolating cytotoxin activity limits the T and B-cell response against H. pylori (9-11).

It was reported, the concomitant presence of cagA+ and cagA− H. pylori organisms in the same patients, and also cytotoxic and non-cytotoxic H. pylori strains were reported at the same time in the same biopsy samples. Our data also suggest that mixed infection with vacA+ and vacA− H. pylori strains is found in Iranian patients which could allow for this bacteria to establish a persistent infection (16).

The epidemiology of H. pylori has been extensively studied during the past two decades. The majority of investigations have utilized endoscopic diagnosis, CLO-test, breath test, serology, and molecular survey of the H. pylori bacteria to delineate the natural history and clinical epidemiology of the associated diagnoses (17). Therefore, serum IgG antibodies to H. pylori, especially, the CagA antigen may be a reliable marker for selection of dyspeptic patients for upper endoscopy.

To our knowledge this is the first study in Iranian patients. The study could have resulted differently it was solely based on vacA alleles. The measurement of serum IgG anti VacA
antibody, and small number of patients are limitations of this study.

In conclusion, the serological response to \( H. \) pylori is heterogeneous, but serum IgG anti-CagA antibodies alone could select patients with dyspepsia for upper endoscopy, therefore assessment of vacuolating cytotoxin activity of \( H. \) pylori was not required, even though \( vacA \) gene was positive. This hypothesis is required to study in a large number of patients with dyspepsia.

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**Conflict of Interest**

The authors declare no conflicts of interest.

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