Risk of cagA DNA in H. Pylori Patients

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Background: Infection of Helicobacter pylori exists all around the world. This bacterium has an IV type secretion system. The main objective of this study was to investigate the existence and abundance of cagA gene in biopsy and serum samples by applying the PCR technique and assay of Triacylglyceride and cholesterol level in sera.

Objectives: The aim of this study was to investigate the correlation between the presence of cagA genome and cardiac risk markers in infected patients with H. pylori.

Patients and Methods: 100 serum samples of patients with above IgG titer against Helicobacter pylori were examined with PCR to investigate the existence of cagA gene. Moreover triglyceride and cholesterol titer and blood pressure were measured.

Results: Eighteen samples out of 100 positive serumic samples from patients with helicobacter pylori had a positive result for the existence of cagA gene. Twelve samples (66.7%) out of eighteen sermic samples had triglyceride and cholesterol titer greater than the normal levels. From 18 specimens detected in sera, about 12 patients had cardiac disorders and 10 patients had high blood pressure.

Conclusions: Since secretion system of type IV is capable of secreting both genome and the protein of this bacterium into the cell, we decided to investigate the existence of cagA gene in sera. This bacterium was unable to induce septicemia and bacteriemia; the possibility is that the gene integrated with protein cagA to blood, was protected from degradation which increases the risk of antibody production against these factors and elevates the risk of heart disease. For this reason, for the first time in the world, we studied the presence of cagA genome in serum samples.

Keywords: Helicobacter pylori; DNA Modification Methylase StyLT1; Risk

1. Background

Helicobacter pylori are gram negative, microaerophilic and lophotrichous bacilli that colonize the stomach of more than half of the world’s population. This bacterium is one of most important agents causing gastritis, adenocarcinoma and peptic ulcer (1-4). Infection with Helicobacter pylori exists all around the world and its prevalence in developing countries has been reported at up to 90 percent. Nowadays, there is a lot of evidence showing that Helicobacter pylori have an etiological role in patient’s digestive system. This bacterium has an IV type secretion system (5).

H. pylori strains have been divided into types I and II. Type I strains express cagA and VacA but Type II strains lack these factors (6, 7). Researchers have identified that patients infected with type I strains are more sensitive to atherosclerosis, adenocarcinoma, peptic ulcer, and gastritis (8, 9).

The cagA protein is one the most important immunogenic proteins in these bacteria. Also, cagA gene causes the induction of c-jun and c-fos oncogenes delays wound recovery, induces IL-8 and increases E2 prostaglandin and stimulation and development of hypertrophy adenoton- silar. These type I strains are associated with atheroscle- rosis, cardiac ischemia and defects of corner vessels. Most eastern strains have cagA and therefore adenocarcinoma is more common in people of east-Asia than the western countries.

Type IV secretory system (T4SS) is effective in proteins and DNA exchange to the outer environment. So far, 7 secretory systems in different negative bacteria have been described and only T4SS enables secretion of DNA molecules to the host cytosol (10, 11).

The cag pathogenicity island (PAI), a 40-kb DNA region, and secret cagA protein and cagA genome into blood stream. Several studies have reported an increase in prevalence of cagA+ H. pylori in type I stains, in gastric cancer (12). Cotemporary to secretion of cagA in gastric epithelial cells, it can lead to variations in host cell structures, cell cycle, cytokines release, and gene expression (5). The cagA protein is more common in East-Asian isolates and is associated with more virulence. The active cagA protein can induce antibodies and cytokines in host cells. About

Implication for health policy/practice/research/medical education:
The aim of this study was to determine the correlation between the presence of cagA genome and cardiac risk markers in infected patients with H. pylori.
60 to 80% of *H. pylori* isolates in the world, express the cagA protein with MW= 120-128 kDa (6). Serologic diagnosis of cagA protein by ELISA is a risk specific indicator for *H. pylori* isolates in infected patients (7). Furthermore, attendance of antibodies to cagA protein in either serum or mucosal secretions is a risk factor (13). Several studies have shown that infection with *H. pylori* cagA+ strains induces damage to gastric epithelial cells and stimulates cytokine secretion that induce inflammatory responses (10, 14).

2. Objectives

The aim of this research was to study the presence of cagA genome as a risk factor for *H. pylori* in sera of infected patients and study the serum markers.

3. Patients and Methods

100 positive serum samples with high serum level for *H. pylori* were collected and stored at −20 °C until assayed. IgG & IgA antibodies against *H. pylori* infection were tested by enzyme-linked immunosorbent assay (ELISA) with curous kit and curous machine. We also assay TG & Chol titers with the biosystem kit and Hitachi machine. Single primer pair was used to amplify the *H. pylori* cagA gene target, 828 bp fragment, based on GenBank. These primers were designed by us and their sequences were as follows; forward primer: ATGACTAACGAAACTATTGATC and reverse primer: TATCGCCAAGAGTGAATTTAG. Serologic detection of triglyceride and cholesterol was carried out according to enzyme linked immunosorbent assay (ELISA) kit and mouse monoclonal antibody isotyping. The aim of this study was to investigate the presence of cagA genome as a risk factor in sera of infected patients with *H. pylori* and correlation this factor has with blood pressure, TG & Chol and cardiac disorders.

4. Results

The measurement of serum titers carried according to the ELISA method and 100 samples that have high titers candidate for this research. *H. pylori* with cagA fragment was amplified by PCR with the above primers. This study indicates that for 12 from 18 positive cagA serum samples, rate of TG & Chol titers were higher than the normal levels and 10 patients had high blood pressure. The results indicate that 12 patients from 18 with cagA in their serum had cardiac disorder predisposition (Table 1).

5. Discussion

*H. pylori* is a bacterium that exists all around the globe and expresses cagA surface protein with MW = 120-128 KD. It has been mentioned that these bacteria were unable to induce septicemia and bacteriemia, however the secretion system of type IV enables secretion of both cagA genome and cagA protein into the cell (10). Therefore, observing the existence of cagA gene, which is one the most important virulence factors, is of great importance. Perhaps, this genome is kept within the folding of cagA protein and is preserved from damage of nuclease enzymes. If the gene along with the cagA protein is present in the blood, the antibodies against it can enhance the risk of atherosclerosis heart diseases and cancer in different areas of the body. Also, there is a risk for integration of part of the genome in human chromosomes.

Also increase of antibodies against these factors intensifies the risk of heart disease (14). For these reasons, we carried a study to investigate the serum of a group of patients and identified 18 percent had the cagA gene. With regards to the identification of cagA in sera, there is a possibility that the gene via integration and simultaneous discharge of the cagA protein in to the blood, may increase the level of antibodies and in turn elevate the risk of heart disease.

Also the increased levels of cholesterol in patients with the cagA gene present in their serum, suggests that there may be a relationship between these bacteria and increased cholesterol levels and this places patients at a greater risk of atherosclerosis. A characteristic of this protein is high antigenicity and immunogenicity (13). Several studies have shown that anti-cagA antibodies can be detected in patients infected with *H. pylori* that contain cagA proteins (13). The presence of anti-cagA antibodies correlates with aggravate disorders, high TG & Chol, atherosclerosis and patients infected with a cagA positive strain were shown to be
more prone for the development of clinically significant
*H. pylori*-related diseases (13).

Approximately 60% of *H. pylori* isolates in the world possess the cytotoxin-associated gene A (cagA), whose presence is associated with various risk factors. Importantly, infection with cagA-positive strains is highly associated with peptic ulcer disease, lichen plan, cardiac syndrome, atrophic gastritis, adenocarcinoma of the stomach and the risk of developing intestinal metaplasia (13).

In order to search for the presence of cagA gene in serum, we designed specific primers, and successfully amplified the cagA gene of *H. pylori* by PCR. The type IV secretory system secretes cagA protein and cagA genome into host cells (10, 13). A number of studies have shown the presence antibodies against the cagA protein, however so far there has not been any report on the detection of cagA genome in sera of *H. pylori* infected patients. In this study, for the first time in world, we were able to detect cagA genome in the serum of patients infected with *H. pylori*. The presence of the cagA genome in infected sera may lead to increased secretion of antibodies against the cagA protein and integration of this genome into host cell chromosomes. *H. pylori* do not perform bacteremia and septicemia but are capable of cagA protein secretion and cagA genome integration into host cells. Thus risk of integration of cagA genome in to the host cell chromosome and induction of antibodies against it may correlate with various disorders (15-18). This research indicates that from 18 positive cagA samples, 12 samples had high TG & Chol titers and cardiac disorder predisposition. 10 patients had high blood pressure.

In this study for first time, we could detect cagA gene in sera of patients with high antibody titers against *H. pylori*. Furthermore we illustrated that the presence of cagA genome is correlated with increased of TG & Chol titers. About 10 of the 18 patients with cagA present in their serum, had blood pressures greater than 12 (range from 13 to 17). Cases of *Helicobacter bacterium* have been reported from time to time. Helicobacter pylori are the most important representatives of *Helicobacterium*, but whether they can result in bacteremia has rarely been studied (16). In this study, we examined *H. pylori* cagA DNA in peripheral blood sera and gastric mucosa of patients with peptic ulcer and gastritis by polymerase chain reaction (PCR). The investigation indicated that 60-70% of gastric biopsy specimens were positive for *H. pylori* cagA DNA. Our findings suggest that *H. pylori* not only exist in gastric mucosa but can also secrete DNA in peripheral blood, and it is possible for *H. pylori* to result in bacteremia or inject DNA in to blood by type IV secretory system; there is a correlation between cagA DNA in blood and cardiac disorder factors (16-20).

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References


