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The presence of cagA genome in blood of infected patient with *Helicobacter pylori* as new marker

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Helicobacter pylori **is a motile and gram negative bacterium, that has been associated with gastritis, peptic ulcer, and gastric adenocarcinoma. The strains of** ***Helicobacter pylori*** **contain CagA associated with severe infections. To investigate the presence of cagA gene in** ***Helicobacter pylori*** **strains among patients' sera, about 100 serum specimens that had positive antibody against** ***H. pylori*** **were collected. Serum samples were studied using designed primers. The scale of triglyceride and cholestrol serum samples was measured. PCR method was done on sera of** ***H. pylori*** **samples that had antibody titers against** ***H. pylori*** **.The results indicated that 18% from all the serum samples contain cagA DNA. This study indicates that 12 serum samples contain triglyceride and cholesterol (TG & Chol) titers higher than normal scale. According to the findings, it is suggested that the presence of CagA DNA in strains of** ***Helicobacter pylori*** **may play an important role in aggravating disorders. In this investigation we were able to detect** ***H. pylori*** **cagA DNA in patients' sera.** ***H. pylori*** **probably attaches to red blood cell and causes bacteremia, but is also able to secrete CagA protein and cagA DNA to host cells by T4 secretory system. Thus, there is risk of integrating cagA DNA in host cell chromosome and inducting antibodies against it. Between the presence of cagA DNA and increase of triglyceride and cholesterol, there exists rational relation.**

Key words: Detection, cagA genome, blood, marker.

INTRODUCTION

Helicobacter pylori is one of the most important bacteria causing gastric infection which infects more than half of the people in whole world, causing chronic gastritis, ulcer peptic and adenocarcinoma (Bruce and Maaroos, 2008l; Choung and Talley, 2008; Costa et al., 2009; Herrera and Parsonnet, 2009; Maeda and Mentis, 2007). *H. pylori* strains have been divided into types I and II. Type I strains express CagA and VacA but type II strains do not show these antigens (Umeda et al., 2009; Sicinschi et al., 2009). Patients have been told that that to be infected with type I strain means to be exposed to adenocarcinoma ulcer peptic, atherosclerosis and gastritis (Jafarzadeh et al., 2009; Zeaiter et al., 2008). The *cag* pathogenicity island, a 40-kb DNA region secretes cagA genome and CagA protein to blood that may be associated with secretion of virulence factors. Several investigations have reported an increased prevalence of CagA positive *H. pylori* in gastric cancer (Hatakeyama, 2006; Handa et al., 2007). Once *H. pylori* CagA is injected into gastric epithelial cells, it can change the host cells structure, cytokines release, cell cycle, and gene expression (Talebkhan etal., 2008, Suriani et al., 2008). The CagA protein is common in East-Asian and associated with increased pathogenicity. The active CagA induces antibodies. About 60 to 80% of

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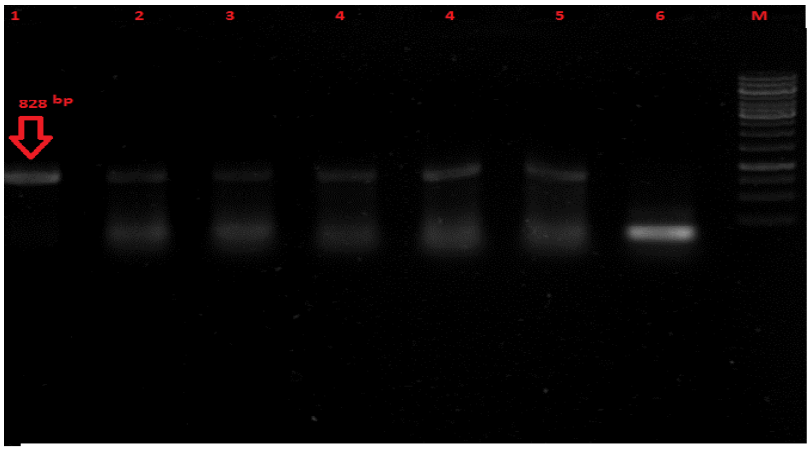

Figure 1. 10 g/L agarose gel electrophoresis of cagA DNA fragment amplified by PCR from *H pylori*. Lane M, Marker 1 kb, Lanes 1, 2, 3 and 4, CagA amplified. Lane 5, Positive control; Lane 6, negative control.

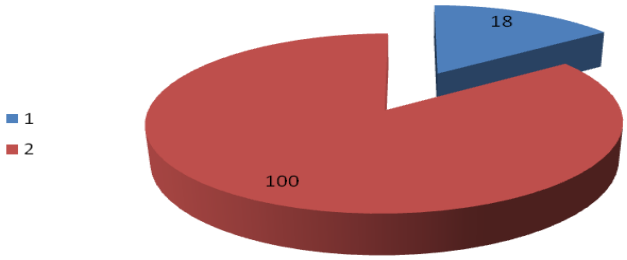

Figure 2. The specimens of positive for cagA genome of *H. pylori* in sera. Number 1 is total specimens and number 2 is positive specimen for *cagA* gene. This figure indicates that from 100 serum specimen, 18 specimens had cagA genome.

H. pylori strains in the world expressed CagA protein with molecular weight of 120 to 128-kDa (Hatakeyama, 2006). Serologic detection of CagA protein by enzyme linked immunosorbent assay (ELISA) is a risk specific indicator for *H. pylori* infection (Cooke et al., 2005). Furthermore, the presence of antibodies in CagA protein in either serum or mucosal secretions is a high risk (Mardh et al., 2002). Several studies have shown that infection with *H. pylori* CagA positive strains induces epithelial gastric cells to secrete increased amounts of interleukin-8 and cytokines that induce inflammatory response of mononuclear and neutrophil cells (Ye et al., 2005; Choi et al., 2007; Esmaeili et al., 2009; Zhang et al., 2006). The aim of this study was to investigate the presence of cagA genome as risk marker in sera of infected patients with *H. pylori.*

MATERIALS AND METHODS

The 100 samples positive with high antibody level against *H. pylori* were collected from Baqyiatallah and Booali hospitals in Tehranand; then were stored at −20°C until assayed. There is ethics regarding the collection and use of the sera in this study. All of the samples were collected after fasting.

IgG and IgA antibodies against *H. pylori* infection were tested by enzyme-linked immunosorbent assay (ELISA).we study sera that had positive seum titers against *H. pylori*. Single primer pair was used to amplify *H. pylori* cagA gene target fragment based on Gene Bank. Primers in early 828 bp of cagA fragment were candidate and were designed with the use of Gene Runner program; considering conserved fragment. These primers were designed by us and their sequences are as follows: Primer forward: ATGACTAACGAAACTATTGATC and Primer reverse: TATCGCCAAGAGTGAATTTAG. PCR was performed in a standard enzyme Taq DNA polymerase concentration of 1 U/50ul reaction, Mg^{2+} 1.5 mM, dNTP 200 uM each; primer, 20pmol/5 0ul; template DNA (5 ul serum) or 50 ng. The mixtures were incubated for 5 min at 94°C for primary denaturation, 1 min at 94°C for secondary denaturation of the target DNA and then, annealing at 55°C for 1 min, and extension at 72°C for 1 min and final extension for 10 min where 35 cycles were performed. The amplified products were analyzed by electrophoresis on 1% agarose gel (cinnagen) containing 0.1 g of ethidium bromide per ml in Tris-borate-EDTA (TBE) buffer. The PCR product was visualized under UV light and photographed. The controls used in the PCR include: negative control being *E. coli* DNA and positive control, cagA DNA from *H. pylori* ss1.

RESULTS

Serum analysis

The serum samples were studied for antibody titers against *H. pylori*. The measurement of serum titer was done according to ELISA kit Mouse Monoclonal Antibody Isotyping. 100 samples that contain high titers antibody against *H. pylori* were candidate for this study.

PCR amplification of *H. pylori* cagA gene

H. pylori with cagA fragment was amplified by PCR from the above primers and the PCR product was electrophoresized and visualized by 10 g/L agarose gel (Figure 1). It revealed that the size of cagA DNA fragment amplified by PCR was 828 bp.The 18% from 100 specimens were positive for cagA genome (Figure 2).

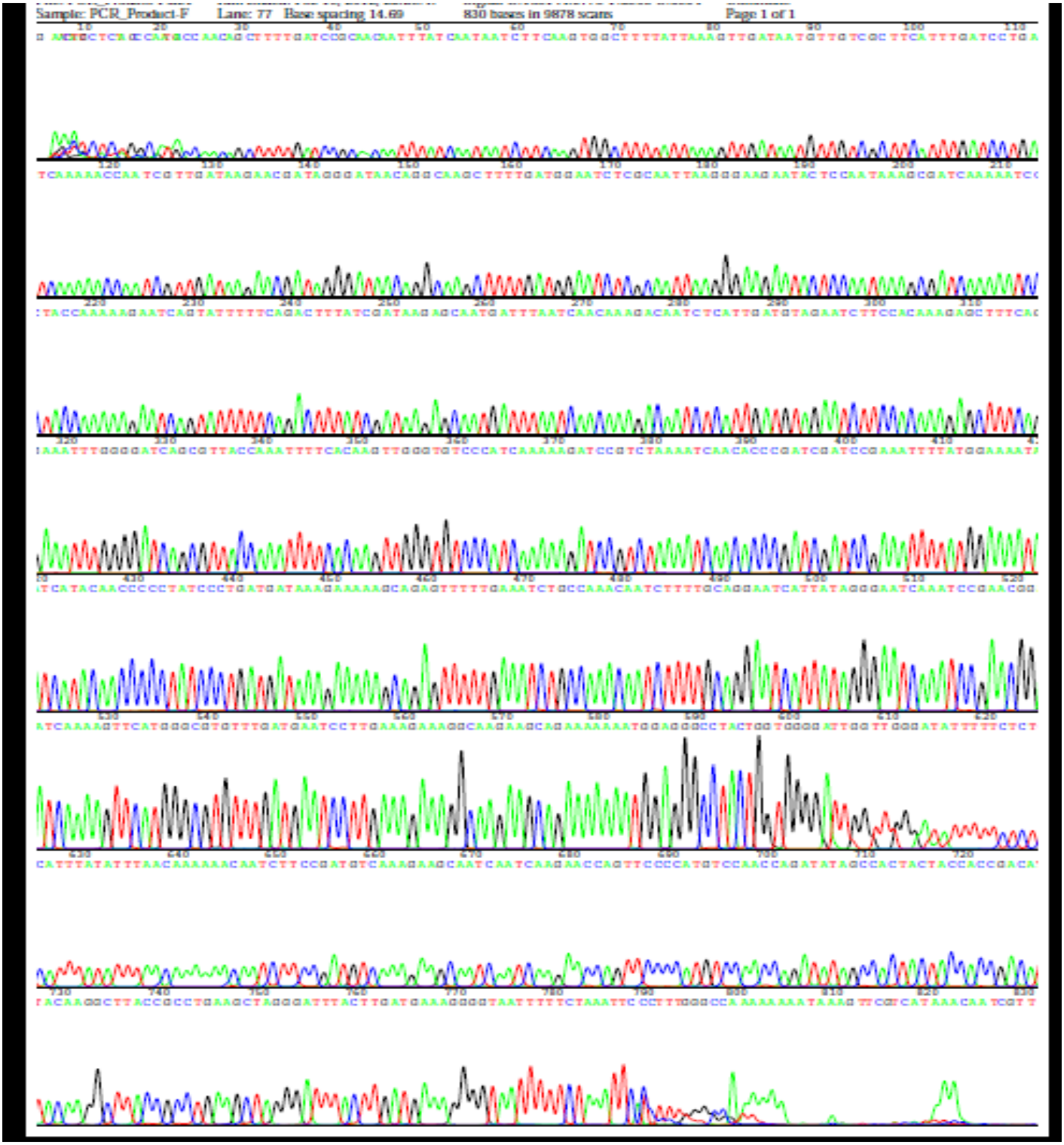

Figure 3. *H. pylori* Sequence of amplified cagA DNA.

Sequence analysis of cagA gene of *H. pylori*

H. pylori Sequence of cagA DNA was analyzed with Forward Primer and primer reverse using automatic sequence analyzer by Sanger dideoxy chain termination method by gene fanavaran. The result of analysis showed that the size of amplified DNA was about 828 bp (Figure 3).

Table 1. The rate of TG&Chol in sera of contains cagA genome in specimens.

Total serum specimen	%
cagA DNA	18%
High TG&Chol titer in total sera	30%
High TG&Chol titer in sra posive with cagA DNA	12%

This table illustrate that from 18 specimen (100%) contain cagA genome there was 12 specimen (66.6%) with high cholesterol and triglyceride titers

Relationship between cagA genome and triglyceride and cholesterol (TG&Chol) titers

This study indicates that 12 from 18 samples with cagA DNA in sera contain TG &Chol titers higher than normal scale (Table 1).

DISCUSSION

H .pylori is a bacterium that exists in the whole world and expresses CagA surface protein with a MW of 120-128 KD. A manifest characteristic of this protein is high antigenicity and immunogenicity (Jafarzadeh et al., 2009). Since the 5'-end of cagA gene contains sequence with conserved regions we used PCR to amplify the 5'-end fragment of cagA with length of 828 bp (Zeaiter et al., 2008). These selective primers are designed for the first time for detection of cagA DNA in serum and biopsy samples.

Several studies have shown that anti-CagA antibodies can be detected in patients infected with *H. pylori* that contain *CagA protein* (Esmaeili et al., 2009). The presence of anti-CagA antibodies correlating with aggravating disorders, high TG & Chol, aterosclerosis and patients infected with a *cagA* positive strain were shown to be more prone for the development of clinically significant *H. pylori*-related disease (Mardh et al., 2002).

Approximately, 60% of *H. pylori* isolates in the world possess the cytotoxin-associated gene A(cagA) and their presence is accompanied with risk factor for adenocarcinoma and atherosclerosis; but in this research we demonstrate 18% from serum samples receptacle cagA DNA. Importantly, infection with *cagA* positive strains is highly associated with peptic ulcer disease, lichen plan, cardiac syndrome, and the risk of developing intestinal metapelasia, atrophic gastritis and adenocarcinoma of the stomach (Mardh et al., 2002). This primer enables the detection of 5/cagA that contains conserved sequences. This primer is specific for conserved regions in all strains. In order to research for the presence of cagA DNA in serum, we designed the specific primers with length of 828 bp based on cagA DNA sequence reported in GenBank, and successfully amplified the cagA DNA of *H. pylori* by PCR. The secretory system type IV secretes CagA protein and cagA DNA into host cells. The number of studies has shown the presence of antibodies against CagA protein but so far does not report detection of the presence of cagA DNA in sera of infected patients with *H. pylori.* In this study, for the first time in the world, we were able to detect cagA DNA in patients' serum infected with H. pylori. Due to the presence of cagA DNA in infected sera many antibodies were secreted against CagA protein and also many probably were integrated into the host cell chromosomes. *H. pylori* does not create septicemia but enables secretion of CagA protein and DNA into host cells and blood. If this bacterium creates bacteremia, it must be able to culture it in synthetic medium. Thus, there may exist risk of integration of cagA DNA into host cell chromosome and induction of antibodies against it that were correlated to several disorders. This research indicates that from 18 samples of positive CagA about 12 samples had titers of high TG& Chol. This study demonstrates that from 100 samples of sera only 18% contain cagA DNA; thus there may be many cagA DNA without CagA protein cleavage in blood by nuclease.

In this study for the first time we can detect cagA DNA in sera of patients with high titers antibody against *H. pylori*. Furthermore, we demonstrate that the presence of cagA DNA is correlated with increase of TG&Chol titers. By in situ hybridization, *H. pylori* bacteria were observed in close association with erythrocytes in capillaries and post-capillary venules of the lamina propria of gastric mucosa in both infected humans and Rhesus monkeys (Aspholm et al., 2006).

Cases of *Helicobacter* bacteremia have been reported from time to time. *Helicobacter pylori* are the most important representative of *Helicobacterium*, but whether it can result in bacteremia has rarely been studied (Huang et al., 2006). 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 investigations indicate that 60-70% from gastric biopsy specimens patients were all positive for *H. pylori* cagA DNA. Our findings suggest that *H. pylori* exists not only in gastric mucosa but also can secrete DNA in peripheral blood, and it is possible that *H. pylori* can result in bacteremia or inject DNA to blood by secretory system type IV.

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