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# Detection of cagA Genome in Sera of Infected Patients to *Helicobacter pylori*

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***Helicobacter pylori*** **has been associated with gastritis, peptic ulcer, and gastric adenocarcinoma. The clinical outcome of *Helicobacter pylori* infection may be associated with or without CagA. To investigate presence of CagA gene in *Helicobacter pylori* strains among patient sera about 100 serum specimens that were positive were taken. serum samples were studied using unique primers, CagA gene was investigated among the *H.pylori* strains. Among tested samples, *cagA* genome was detected in 15. PCR method was done on serum positive samples and were found that the prevalence of CagA gene in patient sera were 18% (18 from 100 cases ). According to the findings, it is suggested that presence of CagA gene in strains of *Helicobacter pylori* may play an important role in aggrevating disorders.In this investigation for first time in world we enable detect cagA genome in patient sera. *H. pylori* dont create bacteremia and septicemaia but enable secret CagA protein and genome to host celles. Thus risk of integration of cagA genom in host cell chromosome and induction of antibodies against it to exist.**

**Key words:** Detection, cagA genome, Serum.

*Helicobacter pylori* is one of common bacteria causing chronic infection, which infects more than half of the human population in all world, causes chronic gastritis and adenocarcinoma[1-5]. *H. pylori* strains have been divided into types I and II that Type I strains express CagA and VacA but Type II strins not express these antigens[6, 7]. It has been recommended patients that to be infected with type I strains more competence to ulcer peptic, Atherosclerosis and gastritis[8, 9]. The *cagA* gene is part of the *cag* pathogenicity island, a 40-kb DNA region, that may be associated with secret of virulence factors. Several studies have reported an increased prevalence of CagA-positive *H. pylori* in gastric cancer[10, 11]. Once *H.pylori* CagA injected into gastric epithelial cells, it can alter the host cells structure,cytokines release, cycle, and gene expression[12,13].The East-Asian CagA associated with increased virulence.Inactive CagA enable induce antibodies.The about 60 to 80% of *H. pylori* strains expressed CagA protein with molecular weight 120- to 128-kDa[14]. Serologic recognition of this protein in an ELISA is a highly specific indicator of *H. pylori* infection[15]. Furthermore, the presence of antibodies to CagA protein in either serum or mucosal secretions is associated with the presence of peptic ulceration[16].The secretary system type IV secrete CagA protein and genome to into host cells. The numbers of studies have shown that infection by CagA-positive strains induces epithelial gastric

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cells to secrete increased amounts of interleukin-8, a cytokine that has a critical role in the inflammatory response of mononuclear and neutrophil cells[17-20]. The aim of this study was to investigate presence of cagA genome in sera of infected patients to *H.pylori.*

## MATERIALS AND METHODS

**Serology**

100 Serum samples that were positive for *H.pylori* were obtained and stored at -20°C until assayed. IgG &IgA antibodies against *H.pylori* infection were tested by enzyme-linked immunosorbent assay (ELISA).we study sera that have positive seum titers.

**Synthetic primers**

Single primer pair was used to amplify *H. pylori* cagA gene target fragment based on GenBank. Primers in early 828 bp of cagA fragment were candidate and were designed with use of DNA star program , consider conserved fragment and absence of EPITY motif. These primers was designed by us and their sequences as follows Primer forward : ATGACTAACGAAACTATT GATC and Primer reverse : TATCGCCAAGAGTG AATTTAG.

PCR was performed in a standard enzyme Taq DNA polymerase concentration 1 U/50ul reaction, $Mg^{2+}$ 1.5mM, dNTP 200 uM each,primer 20 pmol/50ul , template DNA( 5 ul serum) or 50 ng. The mixtures were incubated for 4min at 94°C for primary denaturation, 60 sec at 94°C for secondary denaturation of the target DNA and then, annealing at 55°C for 60 sec, and extension at 72°C for 60 sec that 35 cycle was performed. The amplified products were analyzed by electrophoresis on 1% agarose gel(cinnagen) containing 0.1 g of ethidium bromide per ml in TAE buffer. The PCR product was visualized under UV light and photographed.

## RESULTS

**PCR amplification of *H pylori* cagA gene**

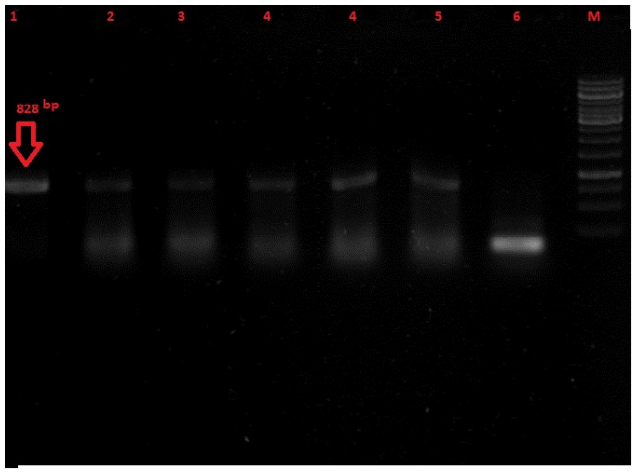
*H pylori* with cagA fragment was amplified by PCR from the above primers and The PCR product was electrophoresed and visualized by 10 g/L agarose gel (Fig. 1). It revealed that the size of cagA DNA fragment amplified by PCR was 828 bp.The 15% from 100 specimens were positive for cagA genome (Fig. 2).

**Sequence analysis of cagA gene of *H. pylori***

*H pylori* Sequence of cagA DNA was analyzed with Forward Primer and Primer primer riverse 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 (Fig. 3).

## DISCUSSION

*H. pylori* is bacterium that express surface protein with a MW of 120-128 KD with name CagA. A dominant characteristic of this




Lane 5:Positive control,Lane6:Negative control, LaneM: Marker 1 kb, Lane1,2,3,4: CagA amplified

**Fig. 1.** 10 g/L agarose gel electrophoresis of cagA DNA fragment amplified by PCR from *H pylori*

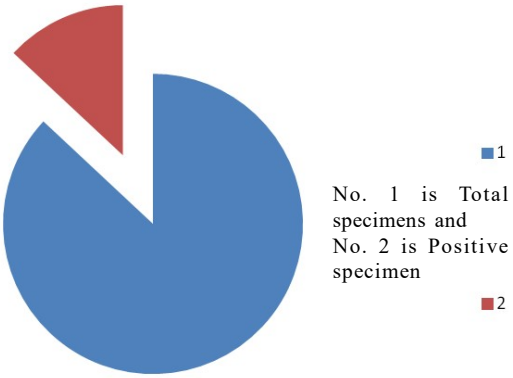



**Fig. 2**. The specimens of positive for cagA genome of *H. pylori* in sera

protein is high antigenicity[8]. Since the 5′-end of cagA had contain sequence with properties of conserved regions we used PCR to amplify the 5′-end fragment of cagA[9].

Several studies have shown that anti-CagA antibodies can be detected in patients infected with a *cagA* positive *H. pylori* strain[19]. The presence of anti-CagA antibodies correlates with aggravate disordres, and patients infected with a *cagA* positive strain were shown to be more prone for the development of clinically significant *H. pylori*-related disease[16].

Approximaetly 60% of *H.pylori* isolates in the world possess the cytotoxin-associated gene A(cagA). Importantly, infection with *cagA*-positive strain is both highly associated with peptic ulcer

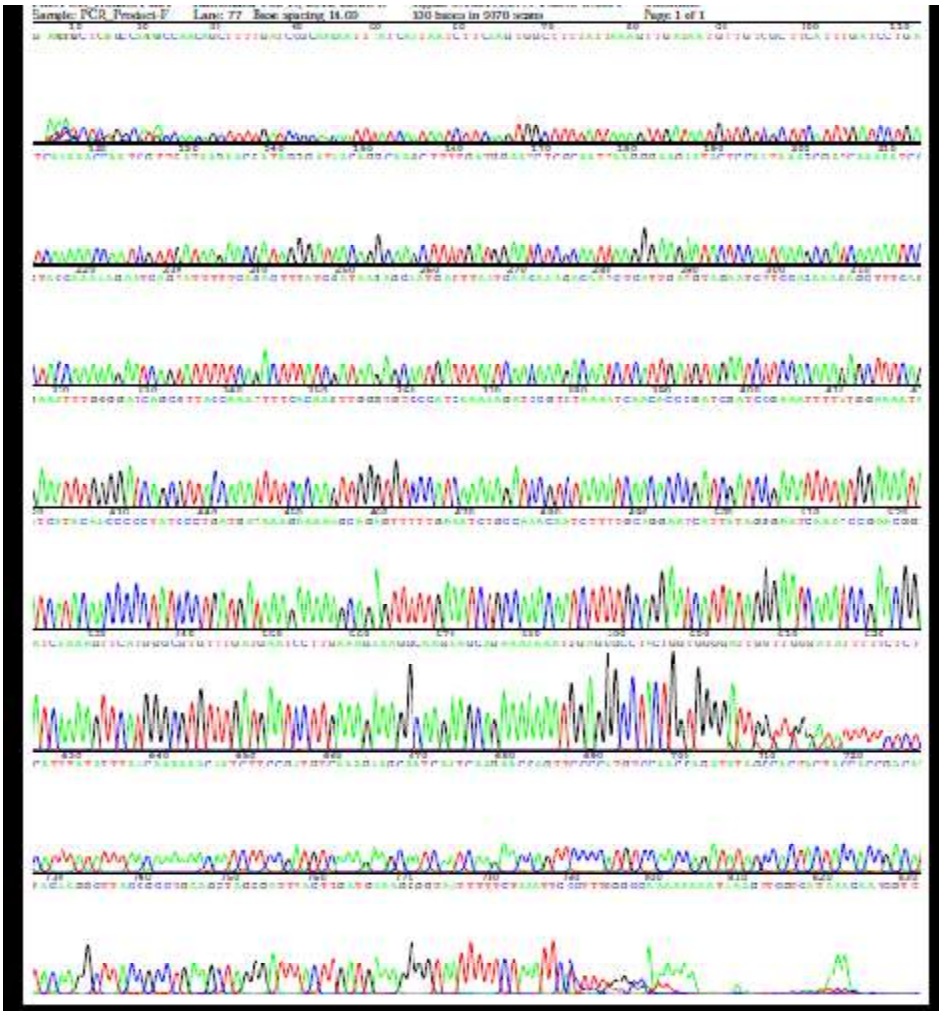


**Fig. 3.** *H pylori* Sequence of amplified cagA DNA

disease, and the risk of developing intestinal metapelasia, atrophic gastritis and adenocarcinoma of the stomach[16].

In order to research the cagA gene' existence in serum we designed the specific primers based on cagA gene sequence reported in GenBank, and successfully amplified the cagA gene of *H.pylori* by PCR. The secretary system type IV secrete CagA protein and cagA genome to into host cells. The number of studies have shown presence antibodies against CagA but so far don't reported detection of presence of cagA genome in sera of infected patients to *H.pylori*.In this investigation for first time in world we enable detect cagA genome in patient serum. Because of cagA genome in serum possible that antibodies secret against it and also may was integrated to into host cell chromosomes. *H. pylori* don't create bacteremia and septicemia but enable secret CagA protein and cagA genome into host celles.Thus may exist risk of integration of cagA genom in host cell chromosome and induction of antibodies against it that may were correlated to several disorders.

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