BioGecko

Vol 12 Issue 03 2023

ISSN NO: 2230-5807

Detection of virulence genes of Helicobacter pylori in infected patients

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Abstract

Helicobacter Pylori is a gram-negative bacterium. It produces urease, has a spiral like conformation, microaerophilic and motile because of the flagella. H.pylori has developed mechanisms to survive the stomach environment and achieve persistent colonization. It is an active stimulator of both the innate and acquired immune responses. The hallmark of H.pylori infection is a marked inflammatory response with the infiltration of various immune cells into the infected gastric mucosa; the host immune response is unable to clear the infection and may actually contribute to the associated pathogenesis.

A total of 150 patients (70females and 80 males), aged between 5 and 60 years were screened for this study. Patients attended the Gastroenterology Unit at AL-hussien teaching hospital in Samawa city from 1st October to1st April 2023, because of recurrent abdominal pain and other gastrointestinal complaints. Three biopsies were obtained from grossly inflamed areas of the antrum. One biopsy was used for Ultra Rapid Urease test and molecular assays to detection of ureB and dup A of geneOur results shows out of 150 patient only 85 (56.66%) patients infected with H.pylori. molecular assays to detection of ureB and dup A of genes for 20 biopsy showed only 13 samples positive for ureB gene and 9 samples were positive for dup A gene

Key word. virulence genes, *Helicobacter Pylori* and Samawa hospital

Introduction

Before the scientists Warren and Marshall, isolated *Helicobacter pylori* from mucosal specimens of patients with chronic active gastritis and peptic ulcer in 1983, the disease was attributed to stress, dietary factors and injurious effects of digestive secretions such as gastric acid. *H. pylori* is a gram-negative, spiral bacterium found in the human stomach, and is one of the most successful human pathogens, infecting approximately 50% of the world's population (1,2). *H. pylori* infection has been identified as a major risk factor for the development of peptic ulcers, gastric adenocarcinoma, Mucosa-Associated Lymphoid Tissue (MALT) lymphoma and other non-gastrointestinal diseases (3,4). *H. pylori* has a number of virulence factors that have been implicated in its avoidance of the immune response and help it to persist in the host. *H. pylori* strains have been traditionally classified into two types on the basis of the Vacuolatingcytotoxin (VacA) and Cytotoxicity associated gene pathogenicity island (PAI) virulence factors (5). Type 1 strains secrete an active form of the vacuolatingcytotoxin and possess the *cag*PAI, whereas type 2 strains secrete an inactive form of VacA and do not possess the *cag*PAI (6,7). This classification of *H. pylori* has also been correlated with clinical presentation. Type 1 strains are typically isolated from persons with severe pathology such as peptic ulcer or gastric cancer, and type 2 strains tend to be isolated from individuals with asymptomatic gastritis (8,9). *H. pylori* infection

provokes a vigorous humoral and cellular immune response in humans, but the organism is rarely eliminated from the gastric mucosa and infection persists lifelong in the absence of treatment (10,11). *H.* pylori colonizes the human stomach and is usually found either as an extracellular pathogen in the gastric mucosa or tightly attached to the cells of the gastric epithelium. Colonization by *H. pylori* results in the release of a range of inflammatory mediators (e.g. interleukin (IL)-8, IL-1*β* , IL-6, interferon (IFN)-ɣ , tumor necrosis factor (TNF)-α, and cyclooxygenase (COX-2) which recruit monocytes, macrophages, neutrophils, and other lymphocytes to the site of infection (12,13).

Materials and Methods

Patients

This study included (150) patients who were referred to AL-Hussien teaching hospital during a period of four months from February 2023 to April 2023. The patient's age ranged from 6-65 years.

3.4.2 Specimen collection:

Blood samples

One hundred and fifty Blood samples were collected from all the studied groups. Patients sera were screened for the presence of *H.pylori*IgG antibodies, Blood samples were collected immediately after endoscopy, and then centrifuged for 15 min. at 1500 rpm; the sera were stored at -20^{o}C until further analyses were performed .

3.2.2: Biopsy samples

Fifty biopsy samples were collected. Endoscopy was performed under local anesthesia forceps were washed with water and disinfected with 2% glutaraldehyde (cidex) for 20min. and then washed with distilled water .two biopsies were used for bacteriological investigation (Rapid urease test and Culture). The two biopsies were transported to the laboratory in 0.5ml –brain heart infusion broth with ice and kept at 4^{o}C.

PCR reaction for virulence genes

DNA extraction

DNA extraction of bacteria from biopsy

G- spin dna extraction kit , intron biotechnology , cat.no. 17045 G- spin dna extraction kit , intron biotechnology , cat.no. 17045

The primers used in PCR reaction

The specific primer **ureB** of gene •

The specific primer **dup A** of gene

Forward	5'-AGTAGCCCGGTGAACACAACATCCT- 3'	62.0	52.0	645 base pair
Reverse	5'- ATGCCTTTGTCATAAGCCGCTTGG- 3’	60.2	50.0	
Primer	**Sequence**	**Tm (^{o}C)**	**GC (%)**	**Product size**
Forward	5'- TGAGCGTGGTAGCTCTTGAC - 3'	56.9	55.0	584 base pair

Reverse	5'- GAGCGCGTTAGCGATATAGG - 3’	55.3	55.0	

Statistical analysis

was exhibited by expending Chi-square ($\chi 2$) examination to control the numerical variations between various collections by consuming **an** application statistical stand for social science (SPSS 19). The opportunity of ($P \leq 0.05$) was restrained to be statistically important.

RESULTS AND DISCUSSION

Distribution of *H.pylori* infection in patients according to the sex.

From results shows that 45(54.11%) male were infected with *H.pylori* and 40(47.05%) from 40 female were *H.pylori* positive. There is significant difference $p<0.01$ was detected between male and female. In spite of the small number of patients, which were studied in this research, the result showed the distribution of *H.pylori* infection in male and female were equally affected by the infection.

molecular detection of virulence genes for *Helicobacter pylori*

From results of 20 biopsy samples of acute cases for *Helicobacter pylori* patients infection

The molecular assays shows 13 samples were positive only for **ureB** gene , while 9 samples positive only for **dup A** gen

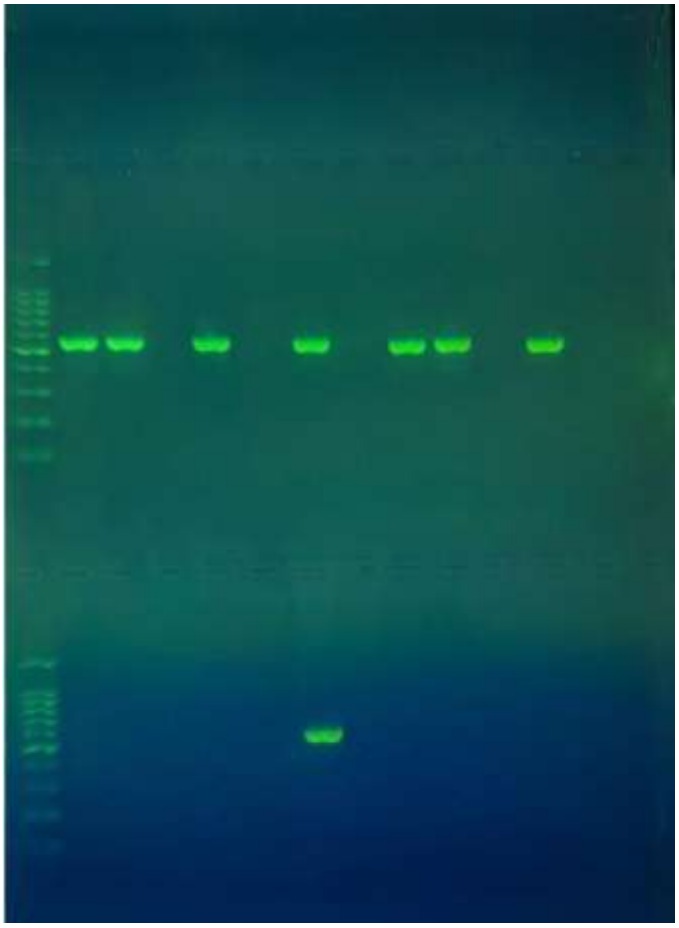

Figure (1) PCR product the band size 584 bp. The product was electrophoresis on 2 % agarose at 5 volt/cm^2. 1x TBE buffer for 1 hr. N: DNA ladder (100).

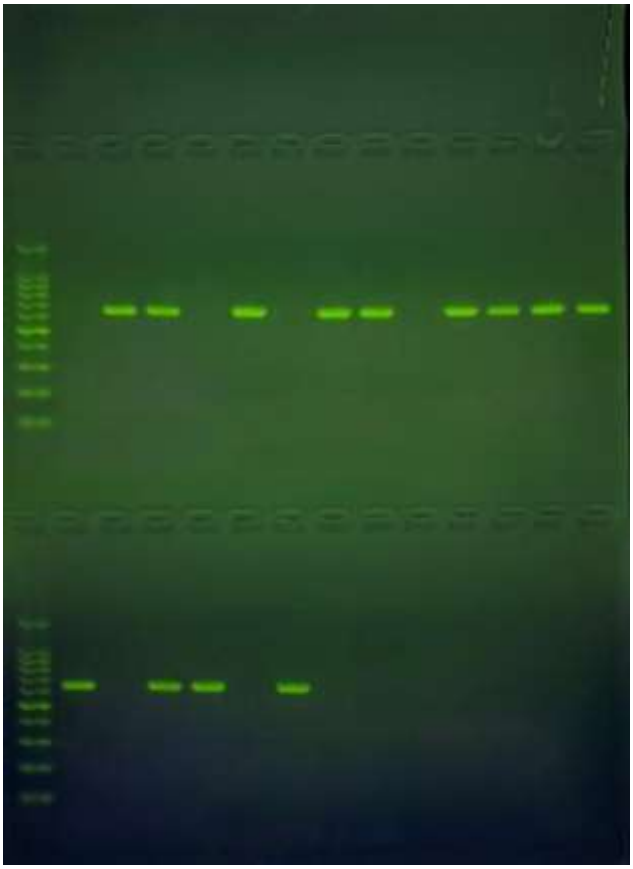

Figure (2) PCR product the band size 645 bp. The product was electrophoresis on 2 % agarose at 5 volt/cm^2. 1x TBE buffer for 1 hr. N: DNA ladder (100).

In the present study the results revealed that *H. pylori* infection was found present in all age groups . The prevalence rate of *H. pylori* infection was more with the mean age of 34. In various developing countries, more than 80% of the population is *H. pylori* positive, even at young ages (15,16).

(17) found that in developed countries, a progressive increase in prevalence is observed, from a low percentage of infection in children to 40 to 50% infection rates in the older age groups. The incidence of new *H. pylori* infections among adults in the Western world is less than 0.5% per year; the higher prevalence of infection among the elderly thus reflects a birth cohort effect with higher infection rates in the past (18). In southern Brazil Santos et al., found that *H.pylori*infection is as common among adults as it is in other developing countries (19).

Most studies suggest that males and females are infected at approximately the same rates as in the current study .(You *et al*., 1998; Hussein *et al*., 2008). Also it was found no association between *H.pylori*infection and gender (p>0.05). Corresponding to the study of (20) found that gender, was not significantly associated with *H. pylori* infection, although in one study by (17) who found male sex was a significant risk factor for infection.Despite the fact that *H. pylori* infection is a mucosal infection, a constant systemic immune response is induced and, because of the chronic nature of this infection, enzyme linked Immunosorbent assay (ELISA) can be used to detect the IgG antibodies produced. The problem with serology is that after eradication of the bacterium, IgG decreases slowly and only after approximately 6 months will a decrease of 50% in the antibody titre be observed (21**).**

The conventional PCR failed to detect the ureB and dup A genes of the *H.pylori*in the extracted biopsy specimens, this could the DNA was damaged during fixation and embedding procedures and requires specific DNA isolation and PCR amplification protocols (22).

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