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Gene detection of *Helicobacter-pylori* by use real-time PCR in patients from Wasit province: Iraq

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Abstract
The present study was conducted to detect the Cytotoxin–associated gene A (CagA) specific for *Helicobacter pylori* by using PCR technique in gastric biopsy of patients in Wasit province at OGD (esophago gastroduodenal scope) Unit at Al-Zahraa Teaching Hospital in Wasit province upper endoscopy for the period from November 2016 to February 2017. Helicobacter pylori colonization with has been recognized as an important risk factor for a gastroduodenal disease accordingly, collected from 40 adult patients, 23 men and 17women, whose age range from 13 to 72 years suffer from dyspeptic symptoms. Referred to the OGD (esophago gastroduodenal scope) Unit at Al-Zahraa Teaching Hospital in Wasit province upper endoscopy for the period from November 2016 to February 2017. According to endoscopic diagnosis, the patients were grouped into gastritis, inflammation of the duodenum, stomach ulcers. Results of analysis of biopsy samples (in size 300mg) by polymerase chain reaction (RT PCR) as invasive procedure of the 40 enrolled patients were subjected for Cag-A gene, result showing seventy-five (75%) (30) patients exhibited positive results to Cag-A gene, also show various ratio in gastritis, gastritis & duodenitis, and gastropath ulcer with positive samples for qPCR-CagA, were ratio (40%), (85.71%) and (87.5%) respectively, the difference in the distribution of H. pylori CagA positive with sex at being nonsignificant (P> 0.05), also no significant was found between CagA and age (P> 0.05).

Keywords: *Helicobacter- pylori*, gene detection, real time PCR, biopsy

1. Introduction

Helicobacter. pylori was classified as a type I carcinogenic agent in humans and is the single most important risk factor in the development of gastric cancer and the second most common cancer worldwide [6]. *H. pylori* infection is one of the most common chronic infections amongst humans since the isolation of the pathogen by Marshall and Warren in 1983 [2]. This organism is different from members of the genus *Campylobacter*, however initially named "*Campylobacter*-like organism," but is now named *Helicobacter pylori* [3]. About 50% of the world's population is estimated to infect by this pathogen [4]. Colonization with *H. pylori* has been recognized as an important risk factor for a gastroduodenal disease, like ulcers, either in the stomach or duodenal, which is one of the most common diseases in recent times [5]. In Iraq there is a serious problem with infection by this bacterium in male and female, it was isolated and identified from peptic ulcer patients by many researchers in Iraq like Al -Baldawy (2001) (for the first time in Iraq) [6], also Twaij, (2006), stated positive *H. pylori* patients by culture of antral biopsy, and by detection of *H. pylori* antigen in saliva [7]. It was reported by Kalaf, (2013) the most important cause of chronic gastritis and gastric cancer, who reported that 102 patients were positive for *H. pylori*, and 100 patient carried different genes 16S rRNA, fla A, ureC and, cagA [8]. The pathogenic potential of *H. pylori* is intricately related to the presence of two most important virulence factors which are encoded by Cytotoxin-associated geneA protein (CagA) and the vacuolating cytotoxin gene (vacA), which is encoded by the cag pathogenicity island (cagPAI) [3]. Different diagnosing methods *H. pylori* bacteria with different sensitivity and characteristics of some invasive process which relies on endoscopy and biopsies, while noninvasive process that do not rely process endoscopy and biopsies are not puppies [9].

Detection of Cytotoxin–associated geneA (CagA) specific for *H. pylori* was conducted in the present study by using PCR technique in gastric biopsy of patients in Wasit province.

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2. Materials and Methods

A total number of 40 patients from 18 male and 12 female were examined by endoscope for *Helicobacter. Pylori* infection, these patients include third age groups: the first one, from 13 to 17 years, the second from 18 to 45 years and the third 46 or more years.

2.1 Samples

A sample of 40 patients with upper gastrointestinal tract diseases, these samples were collected from Al-Zahraa Teaching Hospital in Wasit province for the period from November 2016 to February 2017, Biopsies taking from the greater curve of the gastric antrum by using esophagi-gastro-duodena-scope [10]. The samples were prepared for molecular conformation at the medical microbiology department/ College of medicine/ Wasit University.

2.2 Gastric biopsy specimens

Gastric biopsy specimens (one from each patient) were collected from the stomach antrum or corpus. Specimens were used for extraction of total genomic DNA for qPCR purposes.

2.3 Molecular Detection of *H. pylori*
2.3.1 Bacterial Genomic DNA Extraction

Bacterial genomic DNA was extracted from stomach biopsy samples (100mg/ml) using the manufacturing in genomic DNA extraction kit (Geneaid. USA) [11].

2.3.2 Quantitative Real Time PCR

The *Helicobacter pylori* primers and probes were designed in this study by using the complete sequence of *Helicobacter pylori* CagA gene (GenBank: AB003397.1) from NCBI Gene-Bank data base and Primer3 plus online and provided by (Bioneer company, Korea) (Table 1) (focus on *Helicobacter pylori*. 2009).

Table 1: Primer used in this study.

Primer		Sequence	Amplicon
CagA primer	F	TGCACAACAACCACAAACCG	108bp
	R	TCAGGATCGTATGAAGCGACAG	
CagA probe		FAM-CGGCTTTTAACCCGCAGCAATT-BHQ1	

The qPCR master mix was prepared by using (AccuPrep® 2X Greenstar qPCR Master Mix kit, Bioneer. Korea), and done according to company instructions, the master mix including 5 μL DNA sample (80 ng/μL), 2X Greenstar qPCR master (25μL), each of forward and reverse gene primer (2μL) (10pmol) and DEPC water (16μL) for the total of 50μL. The qPCR strips tubes were placed in centrifuge for 3 minutes at 3000 rpm, and then transferred into Miniopticon Real-Time PCR thermocycler. The PCR thermocycler conditions were set as 1 cycle of initial denaturation 95ºc for 3 min; 45 cycle of denaturation 95ºc for 10 secs; annealing 60ºc for 30 secs, 1 cycle melting 65-95ºc for 0.5 sec repeat 1 cycle [12].

3. Statistical Analysis

Statistical analysis was performed by using SPSS computing program for the analysis of the results [13].

4. Results and Discussion
4.1 Patients Covered Under the Study

The present study included 40 patients; they were suffering from various symptoms of digestive indigestion patients. All the patients were examined endoscopicaly and clinically. The present study results showed the variety of digestive system diseases among the 40 patient: gastritis, inflammation of the duodenum, stomach ulcers in 10 (25 %), 14(35%) and 16(40%) (Table 2).

Table 2: Gastropathological cases of the patients examined by endoscope.

Type of disease	Number of patient	Percent %
Gastritis	10	25 %
Gastritis & doudenitis	14	35%
Gastropath ulcer	16	40%
Total	40	100 %
Chi-square value	2.1	
P-value	0.350	

The comparison of the present distribution of patients with others mentioned in the literature highlights different observations. In this study appeared results variant percentage of pathological cases and was highest percentage in gastropath ulcer about 40% as showed in (Table 2) and this result less difference from Jabbar have found that a distribution 35.56 % gastropath ulcer [14], but agreement with El Sayed Zaki has stated 40.2% of patients with gastropath ulcer [15], and agreement with Abadi has recorded 25% gastritis [16], but disagreement with Kalaf has stated 18.57% with gastropath ulcer [17]. While in case Gastritis & doudenitis present study results disagreement with Busolo *et al.* who has showed 1.04% had both gastritis and duodenitis [18]. It seems that the distribution of diseases different, and might depend on virulence of the infecting *H. pylori* strains, the selection of patients, modulating cofactors, such as smoking and diet the type and the extent of the host immune response to infection [3].

4.2 Diagnosis by quantitative real time PCR

The detection rate of *H. pylori* by PCR technique has shown great agreeing of varying ranging from 0-80% [19].Results of analysis of biopsy samples (Invasive procedure) of the 40 recruited patients (in size 300mg) were subjected to RT PCR analysis for Cag-A gene, Seventy five (75%) (30) Patients exhibited positive results for Cag-A gene, also show various ratio in gastritis, gastritis & duodenitis, and gastropath ulcer with Positive samples for qPCR-CagA, Were ratio respectively (40%),(85.71 %) and (87.5 %) * Significant difference at(p < 0.05). Fig -1- Table -3.
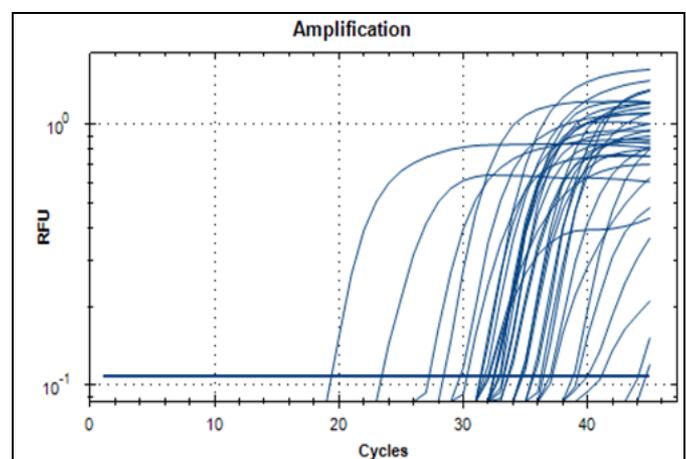

Fig 1: Real-Time PCR Amplification Log Plot of CagA gene in *Helicobacter pylori* from Genomic DNA of Human Biopsy Samples, We're showing the positive detection of *H. Pylori* samples.

Table 3: Relationship between the virulence gene (CagA) of the bacteria *H. pylori* and Pathogenesis cases types.

Type of disease	Number of patient	Positive samples to PCR gene	Percent %
Gastritis	10	4	40 %
Gastritis & doudenitis	14	12	85.71%
Gastropath ulcer	16	14	87.5%
Total	40	30	75 %
Chi-square value	7.097*		
P-value	0.029		

CagA gene 75%, the in the investigated patients less different from Podzoriski *et al.* have stated that CagA-positive gene was detected in 66% of biopsy samples [20] and agreement this percent study recorded by with, Hussein *et al.* have demonstrated 71% CagA –positive [21], Were disagreement with Hadi *et al.* recorded 5.22% CagA-positive [22], this difference between prevalence of CagA -Positive could due to limited number of patients between study [21], Also the geographic location of Kurdistan area CagA- positive (71%) at the crossroads from Turkey which the prevalence of CagA - positive strains in high rates, (92%) [23], thus the description of population may illustrate that genetic exchange has occurred in this region. Also show various ratio depended on pathological cases was the percentage in the case of gastropath ulcer 87.5%, while the percentage in Gastritis & doudenitis and gastritis was around (85.71%), and (40 %) respectively, significant was found between in CagA positive and pathological case tapes (P= 0.029). figure (4-3) which substantiate patients infected with CagA positive strain may be more susceptible to Gastropath ulcer, this agreement with Chen *et al.* has recorded 90.7% present significantly to gastric ulcer compared to other clinical finding [24] and disagreement with El-Sokkary *et al.* has recorded 66.7% [25].

5. Conclusion
Bacterium *H. pylori* was directly detected from biopsy samples of patients using qPCR. The study showed a various relationship between gastropathological cases and *cagA* gene. There was a relationship between the expression of virulence gene and gastric ulcer pathological cases.

6. Acknowledgement

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