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Research Article

Molecular detection of the virulence gene's *VacA* and *CagA* of *Helicobacter pylori* by PCR

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Abstract: *Helicobacter pylori* has several virulence factors, including the ability to produce urease, which allows it to colonize the stomach and survive for long periods of time. As a results of those virulence factors of *H. Pylor*i causes gastritis. This study aimed to detect *H. pylori* using of real-time PCR amplification of *VacA* and *CagA* genes, and compare between both genes and detection by urease and creatine. For this purpose, a total of 50 patients were selected and have been clinically diagnosed with gastritis. Biopsy samples were collected from all the patients. Rapid urease test (RUT) was applied to all gastric samples to detect the presence of *H. Pylori*. DNA was extracted and the RT-PCR was perform to detect the *VacA* and *CagA* genes. The results of RUT showed 20 samples were positive of *H. Pylori*, after the Real Time-PCR test were done the results showed 27 (54%) to *VacA* and 25 (50%) to *CagA* genes were positives.

Keywords: *Helicobactor pylori*, Biopsies, Gastritis, Real-time PCR.

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Introduction

Helicobacter pylori infection causes gastritis, duodenal ulcers, gastric ulcers, and in certain circumstances, stomach cancer. *Helicobacter pylori* is a Gram-negative bacterium that colonizes 50% of human stomachs worldwide (Smith et al. 2019; Haddadi et al. 2020). It has several virulence factors, including the ability to produce urease, which allows it to colonize the stomach and survive for long periods of time. Pathogen has been classified as a type 1 carcino-gen; hence, its persistence in infection without eradication may lead to chronic gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer (Vazirzadeh et al. 2020; Hamidi et al. 2020). To determine the pathogenicity of *H. pylori*, several virulence factors were discovered.

The vacuolating cytotoxin (*vacA*) is of special concern since it prolongs infection (FAO 2013) and has a pleiotropic effect on host cells, including cytotoxicity and apoptosis (Gebert et al. 2003). *VacA* gene is polymorphic in four variable regions, the most characterized are the signal sequence (s), the mid (m) and the intermediate (i), each of these regions show allelic diversity, the s is designated as s1 (s1a, s1b, and s1c) and s2, the m is categorized to m1 and m2, and the i-region consists of i1, i2, and i3 (Isomoto et al. 2010). Another important virulence factor is the cytotoxin-associated gene product (*cagA*) which is encoded on the cag pathogenicity island, is another major virulence factor (PAI). *VacA* is present in all *H. pylori* strains, although only a few are *cagA* positive (Suzuki et al. 2013). *CagA* carriage has been linked to virulence as well as the development of human gastric cancer in studies (Bridge & Merrell 2013). This study aims to detect *H. pylori* using real-time PCR amplification of *VacA* and *CagA* genes, and their comparison.

Materials and methods

The samples of this study were collected in Baghdad teaching hospital, and the gastroenterology and

Table 1. Distribution of all 50 studied patients according to age, gender, and smoking for *Helicobacter pylori*.

		Result		Total	P-Value
		positive	negative		
Gender	Male	1	13	14	0.021
	Female	14	22	36	
Age group	<10	1	0	1	0.271
	11-20	1	3	4	
	21-30	3	10	13	
	31-40	4	14	18	
	41-50	6	6	12	
	>50	0	2	2	
Smoking	smoker	1	12	13	0.039
	Non-smoker	14	23	37	

hepatology teaching hospital from December 2021 to March 2021 from people with age ranged of 10 to 50 years. A total of 50 patients included in this study have been clinically diagnosed with gastritis. Biopsy samples were collected from all the patients.

Detection of *H. pylori*: *Helicobacter pylori* produces urease to transform urea to ammonia and this property is used by a commercially available rapid urease test (RUT) to detect the presence of *H. pylori*. This test was applied to all gastric samples. The tissue biopsies were first put in a tube containing a liquid urea medium and incubated at 37°C for 24 h until the pH urea hydrolysis was examined. As a result, the color of the solution changed from yellow to pink (Blanchard & Nedrud 2012).

DNA extraction from biopsy: Genomic DNA of biopsy samples was extracted by utilizing DNA mini kit (provided by G-spin DNA extraction kit, Korea) using the manufacturer's protocol.

Detection of *VacA and CagA* genes by RT-PCR: The amplification of *VacA* was done using the sense (5'-GGTCAAAATGCGGTCATGG-3') and the antisense (5'- CCATTGGTACCTGTAGAAAC-3') primers, and *CagA* gene using forward and reverse primers of 5'-TTGACCAACAACCACAAACCGAAG-3' and 5'-CTTCCCTTAATTGCGAGATTCC-3' (Van Doorn et al. 1999; Idowu ET AL. 2019). The PCR reaction mixture contains 5µl of pre Master Mix, 1.5µl DNA, 1µl of each forward and reverse primers, then the volume completed to 25µl by deionized water. Thermo cycling conditions were as follows: initial denaturation at 5 min at 95°C, followed by 35 denaturation cycles at 95°C for 45s, annealing at 44°C for 45s, extension at 72°C for 1min and final extension at 72°C for 7min.

Statistics Analysis: Statistical analysis was done using SPSS program (Zaki et al. 2016). The means of the treatments were compared using t-test.

Results

The distribution of study groups by gender, age and smoking habits are shown in Table 1. A total of 50 patients were screened and the result showed positivity in 14(28%) females and 1(2%) male revealing a significant difference ($P<0.021$), however based on age group, no-significant difference ($P>0.271$) was found. Smokers were 13, that 1 (2%) was *H. pylori* positive, and among 37 non-smokers, 14 (28%) were *H. pylori* positive showing significant difference between smoker and non-smokers ($P<0.039$).

The results showed a significantly ($P<0.0081$) higher serum creatinine level in confirmed positive samples (0.8±0.03) than the negatives (0.56±0.1). The urea levels also showed significant higher level

Table 2. Comparison of Biochemical parameters in patients with *Helicobacter pylori* infection.

Results		Creatinine	Urea
Negative	Mean±S.E.	0.56±0.1	28.62±2.7
Positive	Mean±S.E.	0.8±0.03	40.75±2.7
P-Value		0.008	0.01

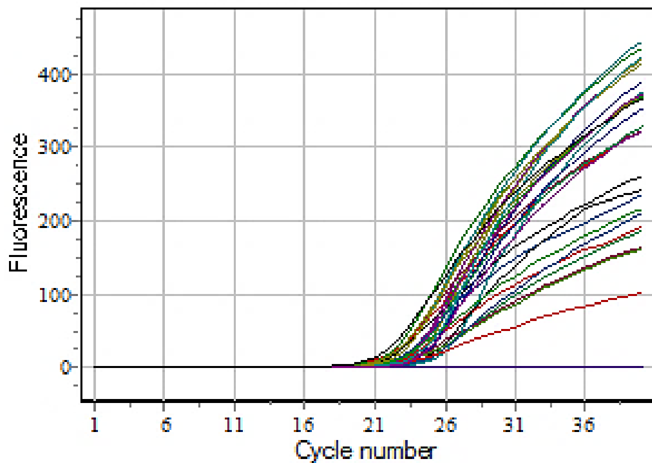

Fig.1. The results of amplification curves of the Vac gene by the RT-PCR**.**

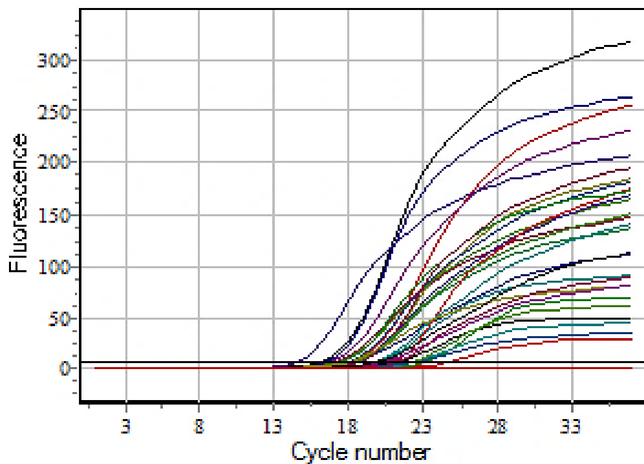

Fig.2. The results of amplification curves of Cag gene by the RT-PCR.

in positive samples (40.75±2.7) than negative ones (28.62±2.7) (Table 2).

The results of RUT showed 20 samples were *H. Pylori* positive, and after the Real Time-PCR, 27(54%) persons were positive for *VacA* and 25(50%) for *CagA* genes (Table 3). The amplification of the *VacA* gene appeared as curves represented by the real time-thermo cycler (Fig. 1), each representing the amplification of single sample. The amplification of the *CagA* gene represented in Figure 2.

Receiver operating characteristic (ROC) curve analysis were performed to investigate the diagnostic values of urea and creatine (Fig. 3, Table 4). The ROC characteristic of the parameters were as follow: area under the curve for urea beta=0.76 (P<0.001; Cutoff=18.0, Sensitivity=100%, Specificity= 100%), and creatine AUC=0.74 (P<0.001; Cutoff=0.235, Sensitivity=100%, Specificity=80%). This results showed that both test can be used as a diagnostic tool for gastritis and specifically the presence of *H. pylori.*

Discussion

In the current study, the biopsy samples were obtained from each patient for rapid urease test and positive results were confirmed by detection of *VacA* and *CagA* genes by RT-PCR assay. The results showed that the majority of *vacA* gene was detected in gastric biopsy samples taken from patients followed by *CagA* gene, and RUT.

The prevalence of *H. pylori* infection (54%) in the studied patients was comparable to Goh's 1997 findings from Malaysia, where the prevalence of *H. pylori* was 49%, and in Kuwait and Saudi Arabia, where the prevalence of *H. pylori* infection was 49.7 and 49.8%, respectively (Alazmi et al. 2010; Hasosah et al. 2015). However, our finding was lower than that reported for Egyptian investigations by Zaki et al. 2016, Ali & Borei 2013, and Abu-Zekry et al. 2013, who found *H. pylori* infection in 60.9, 62 and 70%, respectively. Gastritis and peptic ulcers were found to be 65.2 and 67.3%, respectively, according to Rasheed et al. 2014. *Helicobacter pylori* pathogenesis and disease outcomes are mediated by

Table 3. Percentage of the positive results for each RUT, *VacA* and *CagA* genes amplification

Result	RUT	*Vac gene*	*CagA*
Positive	20 (40%)	27 (54%)	25 (50%)
Negative	30 (60%)	23 (46%)	25 (50%)
Total	50 (100%)	50 (100)	50 (100)

Table 4. The results of receiver operating characteristic curve analysis.

Test Result Variable(s)	AUC	S.E.	Cut off	Sensitivity	Specificity	P-Value	Asymptotic 95% Confidence Interval	
							Lower Bound	Upper Bound
Urea	0.76	0.08	18.00	100%	100%	0.00	0.61	0.91
Creatine	0.74	0.10	0.235	100%	80%	0.01	0.55	0.93

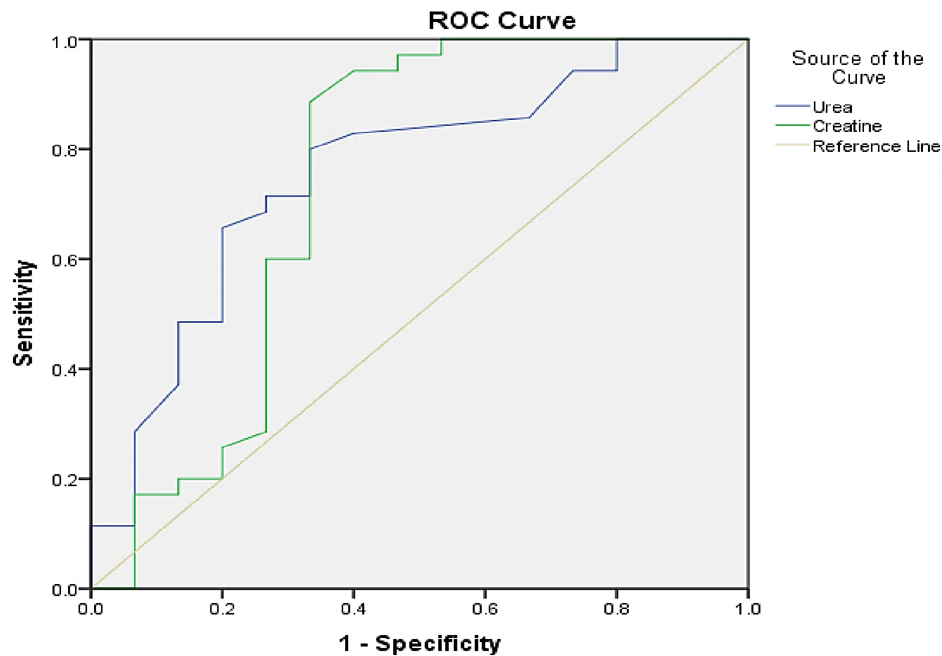

Fig.3. Receiver Operating Characteristic curve (ROC) analysis.

a complex interplay between bacterial virulence factors, host and environmental factors (Kao et al. 2016).

The geographical origin of numerous reports and the results of this study could be a factor in the disagreement. The overall *VacA* gene was recognized in 54% of the *H. pylori* strains investigated, indicating that it is the most virulent gene identified in most of the strains (Havaei et al. 2014; Feliciano et al. 2015), which is consistent with our findings. The variability of the *VacA* gene has previously been linked to geographic regions such as the Middle East, where the frequencies of the *VacA* genotype differed significantly among the southern (Kuwait, Jordan and Saudi Arabia), and northern parts (Turkey, Iran and Iraq). Momenah & Tayeb 2007, Marie et al. 2012 and Kadi et al. 2014 conducted investigations on H. pylori genes in Saudi Arabia, and reported that the general prevalence of *CagA* was 52.4, 62, and 81.8%, respectively (Momenah & Tayeb 2007; Marie et al. 2012 and Kadi et al. 2014). A recent study from Brazil by Sallas *et al.* found *CagA* gene in 50% of

H. pylori isolates (Sallas et al. 2017). Also, similar to our results, was another study from Ecuador reported *CagA* prevalence rate as 45.9% (Sasaki et al. 2009; Sallas et al. 2017). They discovered a link between the *CagA/VacA* genes and gastritis and stomach cancer. In a recent study in Egypt, Abu-Taleb et al. 2018 found that *CagA* was present in 57.4%. *Helicobacter pylori* cagA genes were found in 90% of cases in East Asia (Japan and Korea) and 60%t of cases in North America, Europe, and Cuba (Feliciano et al. 2015). Differences in research sizes, socioeconomic, regional, and genetic factors could explain the wide diversity in *CagA* prevalence rates around the world.

In comparison to neighboring countries such as Kuwait (84%), Jordan (82%), Bahrain (79%), and Egypt (86%), Turkey (63%), Iran (more than 70%), Iraq has a low proportion of *H. pylori* infection (Eghbali et al. 2016). This is thanks to advancements in nutrition, water quality, and antibiotic use. Furthermore; decreasing family size diminishes transmission of *H. pylori*. Many studies have also shown that *H. pylori* infections have been less common in recent years. It is possible that the drop is greater than 26% (Kamangar et al. 2011). Al-Sabary et al. 2017 in a study in Babylon Governorate reported that the prevalence of *CagA* among infected patients was found to be 70.6%, which was similar to the results found in Iraq (71%), Turkey (78%), Europe (70%), and the United States (66%) (Saribasak et al. 2004; Hussein et al. 2008; Nahaei et al. 2008). Besides, *H. pylori* infection is probably involved into chronic kidney damage (Yang et al. 2014) and clinical evidence (Lin et al. 2015). Lin et al. 2015 reported the association between *H. pylori* infection and a subsequent risk of end-stage renal disease (ESRD). One explanation is that the systemic inflammation might play a role in the relationship between *H. pylori* infection and chronic kidney damage. It has been reported that chronic inflammation induced by *H. pylori* may be one of the major causes to renal diseases (Aydogan et al. 2012). Although emerging evidence showed the association of *H. pylori* infection and chronic kidney disease (Subhani et al. 2017; Balat et al. 2019), several studies reported no such an association (Kong et al. 2017; Wijarnpreecha et al. 2017).

As conclusion, we can conclude that targeting *VacA* and *CagA* genes by real time- PCR technique is a good method for the detection of *H. pylori*. In addition, the detection by urease and creatine have a high sensitivity value as it has been shown by the ROC curve analysis.

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