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ORIGINAL ARTICLE

Detection of *vacA*, *cagA* and *iceA* genes of *H. pylori* in dyspeptic patients and their association with clinical data and histopathological abnormalities

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ABSTRACT

Background*: Despite that most H. pylori infections are asymptomatic, some can develop major diseases like peptic ulcer and adenocarcinoma of stomach.* ***Objectives****: This study aimed to study the prevalence of H. pylori using real time PCR. Furthermore, detection of the cagA, iceA1, iceA2, vacA virulence genes and their relationship to clinical and histopathological alterations.* ***Methods****: Two gastric specimens were taken from every patient endoscopically, one of them was sent to histopathological analysis and the second biobsy was minced into tiny parts for real time PCR assay.* ***Results****: The results revealed that the prevalence of H. pylori was 66.67%. Thirty one (50%) of the studied strains harbored cagA gene. IceA gene was positive in 41.94%. The vacA gene was detected in all 62 (100%) samples. Many vacA gene subtypes were detected, the highest found was s1as1bm 20.97%. A significant association was detected between the endoscopic features and vacA presence. The subtypes of vacA: s2, m2,s1a1b, s1as1bm2, were related to gastritis, while s1a, s1b, m1were related to duodenal and stomach ulcers. No significant association between cagA presence and endoscopical or histopathological findings, but more than 66% of duodenal ulcers had positive cagA gene. There was a significant association between the iceA1 and iceA2 genes presence with gastritis and gastric ulcer.* ***Conclusion*** *the study of H. pylori virulence factors allows the clinician to identify high-risk patients caused by H. pylori infections.*

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative pathogen that has a significant association with gastrointestinal disorders development including peptic ulcers, gastric cancer, MALT lymphoma [1,2]. There was an association between gastric cancer and peptic ulcer diseases with more than a million deaths worldwide each year, making it a major public health concern [3,4]. To diagnose *H. pylori* , a lot of procedures are utilized, that can be classified as invasive or non-invasive. The invasive procedures which are the gold standard include microbiological culture, rapid urease test (RUT) and biopsy-based PCR. Noninvasive techniques such as stool antigen testing (SAT), urea breath tests (UBT), and serological tests are well documented and validated [5].

Biopsy-based approaches may suffer from being invasive procedures, bacterial low concentration in fragments, decreased culture sensitivity and error of sampling [6]. Similarly, the accuracy of immunological tests is a point of controversy. Stool antigen assays are inaccurate due to administration of antibiotics, proton pump inhibitor and presence of upper GIT hemorrhage. Therefore, molecular techniques such as PCR have recently improved the diagnostic procedures which detect *H. pylori* . It also detects the genetic alterations in the bacteria to understand drug resistance traits 6 and presence of pathogen co-infection in gastric disorders [7]. The molecular techniques also help in comparative studies between conventional methods like microscopy plus rapid urease test with PCR in low-income regions for proper diagnosis and treatment [8].

The cytotoxin-associated gene A (*cagA*) is found in about half of *H. pylori* strains and is a part of the cytotoxin-associated gene pathogenicity island (*cagPAI*) which is essential for the secretion system type IV. Genotypes of *H. pylori* that are *cagA*-positive cause mucosal irritation and the generation of interleukin-8 (IL-8) and are linked to etiology of stomach malignancy[9]. *CagA* positive has been identified in practically all *H. pylori* isolates recovered from affected individuals in Asian nations [10].

The vacuolating cytotoxin gene (*vacA*) is presented in most of *H. pylori* isolates and codes for vacuolating cytotoxin. It plays a role in the pathophysiology by damaging the epithelial cell of the stomach [11]. The *vacA* gene has two parts: a signal region (*s1/s2*) and a middle

region (*m1/m2*). *VacA* allelic variants in various regions, and their hazardous action, have been documented in prior studies [12]. Another virulence *H. pylori* gene is *iceA* (triggered by epithelial contact) that detected in two allelic forms *IceA1* and *iceA2* , with *iceA1* being extensively linked to peptic ulcer [13,14].

This study aimed to study the prevalence of *H. pylori* using real time PCR. Furthermore, detection of the *cagA, iceA1, iceA2, vacA* virulence genes and their relationship to clinical and histopathological alterations.

METHODOLOGY

Biopsies Collection:

Gastric specimens were collected from 102 cases who had dyspeptic symptom who visited Gastroenterology Clinic in Al Quwayiyah General Hospital, Riyadh, Saudi Arabia. the study start from May 2021 to May 2022 using upper GIT endoscopy, fiberoptic endoscope (EG 530 WR, Fujinon), 2 antral biopsies were taken from every patient. Cases that were excluded from this work were the patients who had taken non-steroidal anti-inflammatory medications, antibiotics or antiacids within the last four weeks before starting the study.

Histopathology examination:

A biopsy from every patient was sent to the regional laboratory, Riyadh to check for histopathological abnormalities caused by the *H pylori* infection. All specimens were fixed overnight using 10% formalin, handled and set in paraffin. Tissue slices which were 4 mm in thickness were obtained then stained using a Giemsa stain (Sheedhan's modified method) and standard hematoxylin and eosin [15] before being analyzed and graded using Sydney's categorization [16].

DNA extraction

It was done by the DNeasy tissue and blood kit (Qiagen, Hilden, Germany) as per the manufacturer guidelines after mincing the specimens into tiny parts with sterile scalpels.

***H. Pylori* Detection by Real time (RT) PCR**

All DNA samples were collected from the 102 gastric specimens and were subjected to amplification by real-time PCR using the kit of *H. pylori* genesig Quantification ((PrimerDesign Ltd. Southampton, U. K), that was based on primer-probe and targets of the *H. pylori* rpoB gene . The assays were done in volumes of 20 µl which included 10 µl of "oasig™ 2× qPCRMastermix" (PrimerDesign Ltd.), 1 µl specific primer/probe of *H. pylori* , 1 µlprimer/probe mix as an internal control, 3 µl of the specimen extracted DNA, 2 µl of DNA internal control, a volume of 20 µl was made up by putting RNase/DNase free water. The PCR reactions were done using Smart Cycler (Cepheid, Italy). During each PCR run the positive control which was DNA of *H. pylori* provided in the kit was added to the reaction and DNase/ RNase free water was also added to the reaction as a negative control. The PCR cycles were 50 cycles of denaturation at 95°C for 10 s and annealing and extension at 60°C for 60 s. [17].

***VacA, cagA* and *iceA* virulence genes detection**

Real time PCR for *H. pylori* was used for *vacA, cagA* and *iceA* virulence genes detection. Every gene was identified using a unique PCR with its own primer pair. The amplification processes were done in a volume of 50 µl using reaction buffer. 5 µl of 10 x PCR buffer enriched with $MgCl_2$ (15 mM $MgCl_2$, 50 mMKCl, 10 mMTris-HCl), DNT mix (0.2 m M of dCT, dATP, dTTP, and dGTP) (Roche, Germany), 2.5 U of Fast Start *Taq* DNA polymerase (Roche, Germany), 0.4 µM of every primer, 5 µl of DNA template, RNase and DNase free water were added up to a volume of 50 µl. The PCR cycling was 30 cyclesfor 10 min of 94 °C then 55 °C for 2 min and 72 °C for 2 min, laterthe extension step, 72 °C for 10 min. Separation of the PCR products was done using Tris-acetate-EDTA buffer and 1.5% agarose gels (Promega, Madison, USA) then staining by ethidium bromide and visualization of bands using a U.V. transilluminator [14].

Table 1: Primers used for *cagA*, *vacA* and *iceA* virulence genes detection by Real time PCR

Primers	Sequence	Product size
CagA-F	GATAACAGGCAAGCTTTTGAGG	349
CagA-R	CTGCAAAAGATTGTTTGGCAGA	
S1a-F	TCTYGCTTTAGTAGGAGC	212
S1a-R	CTGCTTGAATGCGCCAAAC	
S1b-F	AGCGCCATACCGCAAGAG	187
S1b-R	CTGCTTGAATGCGCCAAAC	
S1c-F	CTYGCTTTAGTRGGGYTA	213
S1c-R	CTGCTTGAATGCGCCAAAC	
S2-F	GCTAACACCCAAATGATCC	199
S2-R	CTGCTTGAATGCGCCAAAC	
M1-F	GGT AAAATGCGGTCATGG	290
M1-R	CCATTGGTACCTGTAGAAAC	
M2-F	GGAGCCCCAGGAAACATTG	352
M2-R	CATAACTAGCGCCTTGCAC	
*IceA1*F	GTGTTTTTAACCAAAGTATC	247
IceA1-R	CTATAGCCASTCTTTGCA	
IceA2-F	GTTGGGTATATCACAATTTAT	229/334
IceA2-R	TTRCCCTATTTTCTAGTAGGT	
Cag empty site Luni1-F	ACATTTTGGCTAAATAAACGCTG	550
Cag empty site Luni1-R	GGTTGCACGCATTTTCCCTTAATC	

Statistical analysis

The data was interpreted by the use of SPSS version 20 (IBM Corp., NY, USA). Descriptive and inferential statistics were used in the statistical tests. The Chi Square/Fisher Exact test was used to determine whether proportions differences were significant. To illustrate statistical significance, P-values less than 0.05 was considered significant.

RESULTS

The *H. pylori* was detected in 68 (66.67%) samples of 102 collected biopsies using R.T PCR, Table 2. showed that only 62 (91.18%) of them harbored virulence genes. It was found that out of 62 virulent *H. pylori* strains, 31(50%) were *cagA* positive, 30 (48.39%) were *cagA* negative and 1(1.61%) was mixed. *IceA* gene was detected in 26 (41.94%) while it was negative in 21(33.87%) and mixed *iceA1* and *iceA2* in 2(3.23%). *VacA* gene was detected in the 62 virulent strains (100%). Many *vacA* gene subtypes were detected, the highest found was *s1as1bm* 13 (20.97%) strains followed by *m2*, *s2* and *s1a1b* alleles which were found in 11 (17.74%) strains for each subtype.

Table 2: Genes distribution in *H.pylori* strains among studied patients

Genes identified		No of isolates = 62	Percentage
CagA	Positive	31	50%
	Negative	30	48.39%
	Mixed	1	1.61%
Vac A	*S1a*	6	9.68%
	S1b	8	12.90%
	S2	11	17.74%
	M1	1	1.61%
	M2	11	17.74%
	S1a1b	11	17.74%
	S1as1bm1	1	1.61%
	S1as1bm2	13	20.97%
IceA	*IceA 1*	26	41.94%
	IceA2	21	33.87%
	Mixed	2	3.23%
	Negative	13	20.97%

Table 3. showed the correlation between various *H. pylori* genotypes with age and sex. There was no significant statistical difference between the *cagA*,*vacA* and *iceA* genes presence and gender. Although, m2 subtype of *vacA was* found to be increased in females, 7(63.64%) than males, 4(36.36%), while mixed *iceA* was only found in males.No significant differences were observed between *cagA*, *vacA* and *iceA* genotypes and the various groups of age. However, *m2* was high 6 (54.55%) in the patients aged from 18 to 29 years, while it was decreased 1(9.09%) among patients aged from 30 to 49 years and 3(27.27%) in patients aged more than 50 years old.

Table (3): Correlation between various *H. pylori* genotypes with age and sex

Genes		**Sex**		***P***	**Age**				**Total**	***P***
		Male=32	Female=30		18-29	30-40	40-49	50+		
CagA	Positive	15(48.38%)	16(51.61%)	0.698	11(35.48%)	6(19.35%)	6(19.35%)	8(25.80%)	31	0.229
	Negative	16 (53.33%)	14(46.67%)		12(40.00%)	5(16.67%)	7(23.33%)	6(20.00%)	30	
	Mixed	1(100.00%)	0(0%)		0(0%)	1(100%)	0(0%)	0(0%)	1	
Vac A	S1a	3(50.00%)	3(50.00%)	0.509	2(33.33%)	3(50.00%)	1(16.67%)	0(0%)	6	0.298
	S1b	5(62.50%)	3(37.50%)		2(25.00%)	1(12.50%)	3(37.50%)	2(25.00%)	8	
	S2	6(54.54%)	5(45.45%)		4(36.36%)	3(27.27%)	3(27.27%)	1(9.09%)	11	
	M1	0(0%)	1(100%)		0(0%)	1(100%)	0(0%)	0(0%)	1	
	M2	4(36.36%)	7(63.64%)		6(54.55%	1(9.09%)	1(9.09%)	3(27.27%)	11	
	S1a1b	7(63.63%)	4(36.36%)		1(9.09%)	3(27.27%)	4(36.36%)	3(27.27%)	11	
	S1as1bm1	0(0%)	1(100%)		0(0%)	0(0%)	0(0%)	1(100%)	1	
	S1as1bm2	7(53.84%)	6(46.15%)		2(15.38%)	4(30.77%)	2(15.38%)	5(38.46%)	13	
IceA	IceA 1	13(50.00%)	13(50.00%)	0.531	8(30.77%)	8(30.77%)	6(23.08%)	4(15.38%)	26	0.569
	IceA2	10(47.62%)	11(52.38%)		9(42.86%)	2(9.52%)	5(23.81%)	5(23.81%)	21	
	Mixed	2(100%)	0(0%)		1(50.00%)	0(0%)	1(50.00%)	0(0%)	2	
	Negative	7(%53.85)	6(46.15%)		5(38.46%)	2(15.38%)	3(23.08%)	3(23.08%)	13	

Table 4. showed the correlation between *vacA* with endoscopic features and histopathological findings. A significant association, (*p* value, 0.001) was found between the endoscopic features and *vacA* gene presence. The subtypes of *vacA*: (*s2, m2, s1a1b, s1as1bm2),* were related to gastritis, while, subtypes *s1a, s1b, m1* were related to duodenal and gastric ulcers. No statistical significance (*p* value of 0.229) was detected between genotypes of *vacA* and histopathological alterations, although, *vacA* subtypes: (*s2, ,s1a1b, s1as1bm2)* were increased in severe chronic active gastritis. The *vacA* subtypes *S1a and S1b*a were associated mainly with severe gastritis, while the subtype *vacA,m2 and S1as1bm2* was highly associated with moderate active gastritis *S1as1bm1*

Table 4: Correlation between *VacA* with endoscopic feature and histopathological findings

	VacA								**Total**	**P**
	S1a	*S1b*	*S2*	*M1*	*M2*	*S1a1b*	*S1as1bm1*	*S1as1bm2*		
Endoscopic feature										0.001
Normal	1(11.11%)	2(22.22%)	1(11.11%)	0(0%)	2(22.22%)	1(11.11%)	0(0%)	2(22.22%)	9	
Gastritis	2(4.44%)	2(4.44%)	10 (22.22%)	0(0%)	9(15.56%)	10(17.78%)	1(2.22%)	11(20%)	45	
Gastric ulcer	2(40%)	3(60%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	5	
Duodenal ulcer	1(33.33%)	1(33.33%)	0(0%)	1(33.33%)	0(0%)	0(0%)	(0%)	0(0%)	3	
Histological findings										0.229
Chronic gastritis										
Mild	0(0%)	2(28.57%)	2(20.75%)	0(0%)	2(20.75%)	1(14.28%)	0(0%)	0(0%)	7	
Moderate	2(18.18%)	0(0%)	1(9.09%)	0(0%)	3(27.27%)	2(18.18%)	0(0%)	3(27.27%)	11	
Sever	1(25.00%)	1(25.00%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	2(50.00%)	4	
Chronic active gastritis	3(7.50%)	5(12.50%)	8(20.00%)	1(2.50%)	6(15.00%)	8(20.00%)	1(2.50%)	8(20.00%)	40	

Table 5 showed non significant differences between *cagA* presence and endoscopical or histopathological changes, but more than 66% of duodenal ulcers had positive *cagA*. There was significant association between *iceA1* and *iceA2* genes and gastritis and gastric ulcer as *p* value was 0.027. There was an association between *iceA1* and *iceA2*with chronic active gastritis, 40% and 45% respectively, while the *iceA* negative cases were associated with chronic active gastritis in (15%) only.

Table (5): Correlation between *cagA* and *iceA* with endoscopic feature and histopathological findings

	CagA			p	***IceA***				**Total**	**p**
	positive	negative	mixed		Ice A1	IceA2	Mixed	Negative		
Endoscopic feature				0.479						0.027
Normal	4(44.44%)	5(55.56%)	0(0%)		2(22.22%)	3(33.33%)	0(0%)	4(44.44%)	9	
Gastritis	22(48.88%)	22(48.88%)	1(2.22%)		21(46.66%)	15(33.33%)	2(4.44%)	7(15.56%)	45	
Gastric ulcer	2(40.00%)	3(60.00%)	0(0%)		3(60.00%)	1(20.00%)	0(0%)	0(0%)	5	
Duodenal ulcer	2(66.67%)	1(33.33%)	0(0%)		1(33.33%)	1(33.33%)	0(0%)	1(33.33%)	3	
Histological findings										
Chronic gastritis				0.698						0.481
Mild	4(57.14%)	3(42.86%)	0(0%)		4(57.14%)	3(42.86%)	0(0%)	0(0%)	7	
Moderate	4(45.45%)	7(63.63%)	0(0%)		5(45.45%)	0(0%)	0(0%)	6(54.54%)	11	
Sever	3(75%)	0(0%)	1(25%)		1(25%)	0(0%)	2(50%)	1(25%)	4	
Chronic active gastritis	20(50%)	20(50%)	0(0%)		16(40%)	18(45%)	0(0%)	6(15%)	40	
Total	31	30	1		26	21	2	13	62	

Fig.(1): showed Real time PCR amplified *cagA* gene products (349 bp), *iceA1* gene (247 bp) and *iceA2* gene (229 or 334 bp).

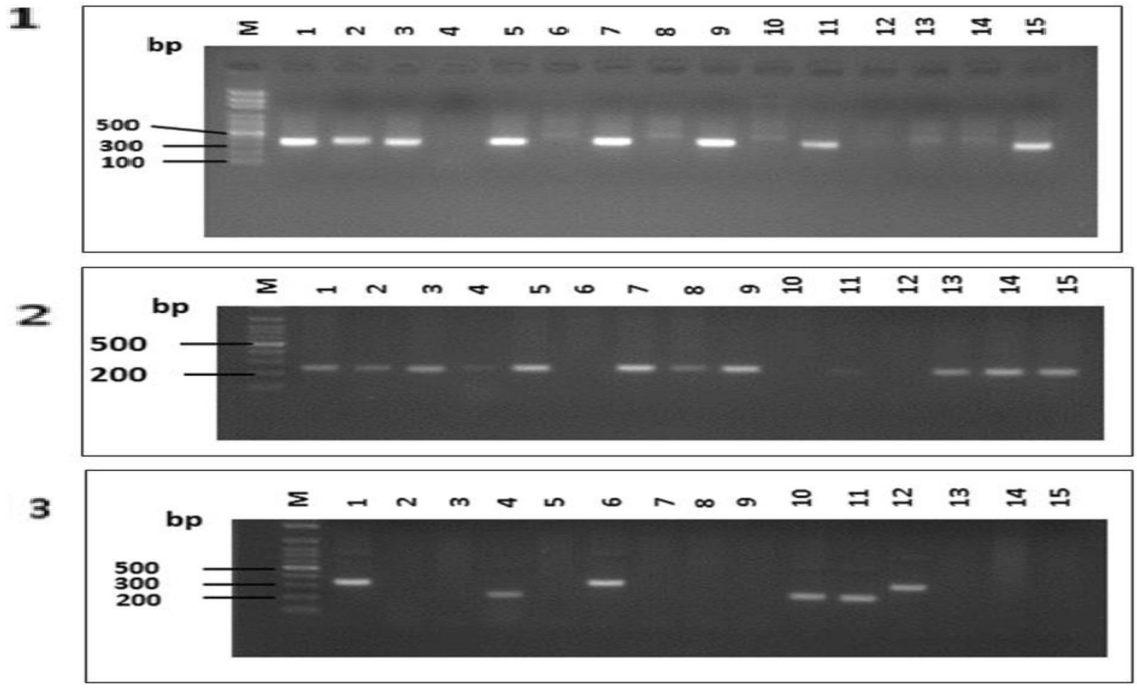

Fig. 1: showed PCR-based genotyping amplified products of (1) *cagA* gene (349 bp).Lanes; M; DNA ladder (100 bp). *cagA* positive observedinLanes 1, 2, 3, 5, 7, 9, 11, and 15 (2) iceA1 gene (247 bp). Lanes; M; DNA marker (100 bp), iceA1 positive was seen in Lanes 1, 2, 3, 4, 5, 7, 8, 9, 11, 13, 14 and 15. (3) ice*A2* gene (229 or 334 bp). Lanes; M; 100 bp molecular DNA marker, Lanes 1, 4, 6, 10, 11, and 12 are *iceA2* positive. The PCR products in lanes 1, 6 and 12 are of 334 bp size, while lanes 4, 10 and 11 have 229 bp PCR products

Fig. (2): showed PCR-based genotyping amplified products of *vacA* gene

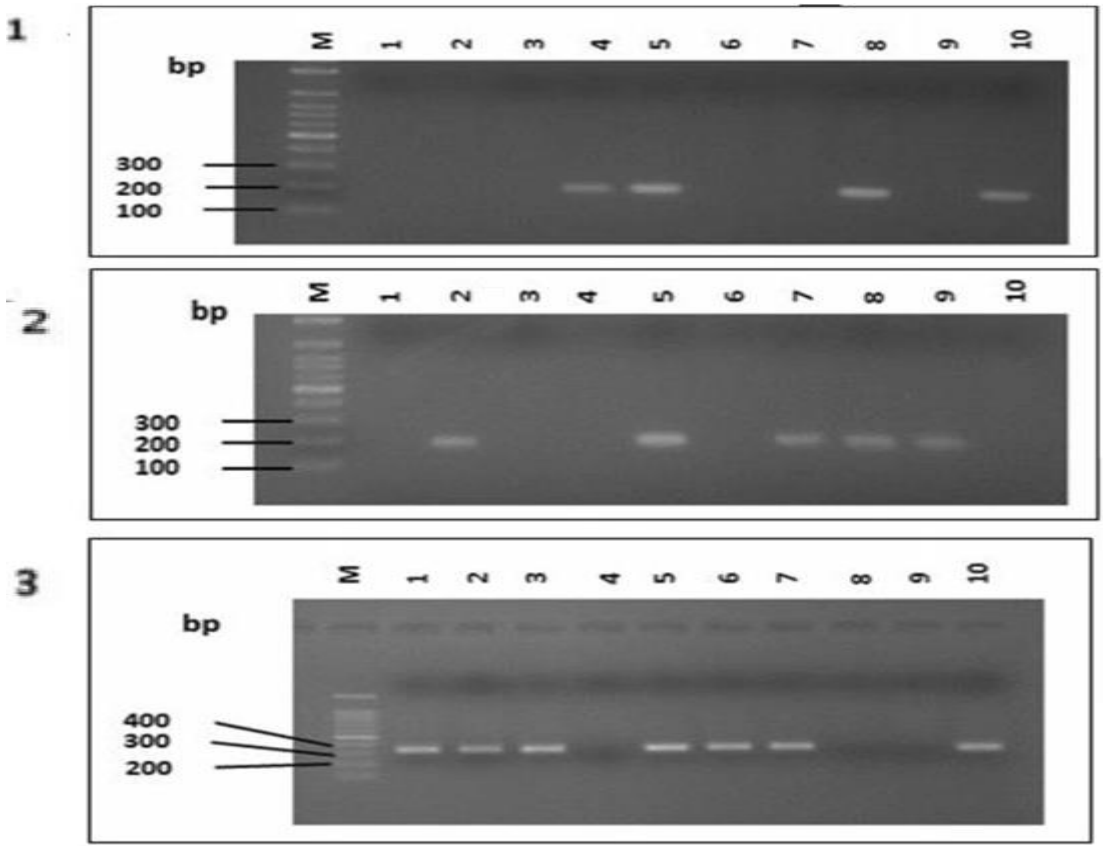

Fig. 2: showed PCR-based genotyping amplified products of *vacA* gene .(1) PCR products of *vacA s1a* gene (212 bp). Lanes; M; 100 bp molecular DNA marker, positive *vacAs1a* gene was found in Lanes 4, 5, 8, 9, and 10 (2) PCR products of *vacA s1b* gene (187 bp). Lanes; M; 100 bp molecular DNA marker,*vacAs1b* gene was detected in Lanes 2, 5, 7, 8 and 9. (3) products of *vacA m2* gene (352 bp). Lanes; M; 100 bp molecular DNA marker , *vacAm2* gene detected in Lanes 1, 2, 3, 5, 6, 7 and 10 .

DISCUSSION

H pylori colonizes the stomach and causes inflammatory response in the mucosa of stomach known as "gastritis." Without antibiotic therapy, such a disease can last for decades. Gastritis is the earliest visible changes in a step-wise process of histopathological changes which might proceed to gastric tumor [18]

In this work *H. pylori* prevalence was 66.67% as tested by real time PCR. This result matched with another study conducted in Al Kharj region in Riyadh by Alanazi et al. [19] who reported *H. pylori* presence among the involved cases was (62.2%). In several researches all-over the world in United States, Brazil and China, the *H. pylori* prevalence between dyspeptic patients was 28.9%, 57%, and 84% respectively Ramis et al.[20] Another study by Aleid et al. [21] reported that the *H. pylori* prevalence in studied cases in Riyadh was 39%. This data was lower than this report. This difference among Saudi Arabia regions was due to several reasons, such as personal hygiene, healthcare system, and socioeconomic status [22]. In this study *CagA* positive were 50%, while 48.39% were negative for *cagA* and 1.61% was mixed. Similar results were obtained by Akeel et al. [17]. who detected *cagA* in 49.2% of *H. pylori* strains . The results also were similar to the studies by Momenah et al. [23], Marie et al. [10]., Kadi et al. [14] in Saudi Arabia, they found *cagA* prevalences were 52.4%, 62% and 81.8% respectively. In Egypt, Abu Taleb et al.[24] reported the prevalence of *cagA* was about 57% in their studied group.

In our study *iceA* gene was harboured in 41.94% of strains, while it was negative in 33.87% and mixed *iceA1* and *iceA2*in 3.23%. Akeel et al.[17] also reported that about 42 % of *Helicobacter pylori* had *iceA1* and 32.8% had *iceA2*. Similar findings also obtained with Sedaghat et al. [25]. Abu Taleb et al [24] who found the prevalence *iceA* gene in 48.6% and 46.29% cases respectively.

In the present work, *vacA* was revealed in the 62 (100%) virulent *H. pylori* isolates. Many *vacA* gene subtypes were detected, the highest found was *s1as1bm2* 20.97%, then *m2, s2* and *s1a1b* alleles which were detected in17.74% strains for every subtype. The same findings were revealed by El Khadir et al.[26] who reported that 99% of *H pylori* isolates in Morocco had *vacA*, while decreased *vacA* gene rates were reported in Ethiopia (90%) and Netherland (93%)[26] . Regarding *vacA* gene subtypes Akeel et al.[17] observed that the highly detected *vacA* subtypes were *s1as1bm2*, *m2* and *s1a1b* which detected in 23.4%,16.4% and 16.4% of *H. pylori* isolates respectively. Similar results were found by Pajavand et al.[27] that the most detected *vacA subtypes* were *m2* (39.5). Moreover, Marie et al. [11] reported *vacA* alleles in Saudi cases as *vacAm2* (30%) and *s1a1b* (16%) in cases suffered from dyspepsia.

In the current work, no significant statistical difference was noticed among the *cagA*,*vacA* and *iceA* presence and gender. Although, *m2* subtype of *vacA* was increased in females, 63.64% than males36.36%, while among males *m1*, it *was* 100*%* and *m2 was* 63.64 %. Kadi et al.[14] also observed no sex variations associated with *cagA* and *iceA* genes distribution, while they reported that some *vacA* genotypes observed in female gender as *vacA m2* subtype in percentage of 65%, while it was 35% among men. Similar to Like these findings, El Khadir et al.[26] reported that *vacAm2* was more in women than in men.

Our work showed no observed significant differences between *vacA*, *cagA* and *iceA* and various groups of age. However, *m2* was high (54.55%) in the patients aged from 18 to 29 years, while it was decreased 1(9.09%) among patients aged from 30 to 49 years. and 3(27.27%) in patients aged more than 50 years old. Akeel et al.[17] also reported that *vacAm2* subtype was more prevalent (62%) in young patients aged from 13 to 29 years, but in older patients, this gene prevalence was decreased (about 24 and 14 % in the patients aged between 30 and 49 years and more than 50 years, respectively). Also similar results were mentioned by Feliciano et al.[28].

In the present work a significant association (*p* value, 0.001) between the endoscopic features and *vacA* gene presence. The subtypes of *vacA*: *s2, m2,s1a1b, s1as1bm2,* were related to gastritis, while, *s1a, s1b, m1* subtypes were related to duodenal and gastric ulcers. These results matched with Pajavand et al. [27] and Sallas et al. [29] who reported association between *s2 and m2* with gastritis and association between *s1 and m1* with gastric ulcer. A research in Saudi Arabia conducted by Bibi et al. [30] revealed increased prevalence of *vacA s1* in patients who suffered from gastric ulcer (80%) or cancer (100%). Many researches were done in Western countries, Africa, China and Middle East, which reported that patients who harbored *vacA s1* or *m1 in H. pylori* strains had more risk for development of peptic ulcer and gastric cancer if compared with patients who had *s2* or *m2 H. pylori* isolates. According to these findings *vacA s2* and m2 isolates appeared to be less virulent than *s1a, s1b, m1* strains [31]

In this study no statistical significance (*p* value of 0.229) was detected between genotypes of *vacA* and histopathological alterations, although, *vacA* subtypes: *s2, ,s1a1b, s1as1bm2* were increased in sever chronic active gastritis. Akeel et al. [17] also reported an increased rate of *vacA s1a1b* (16%). This finding matched also with the research of Sedaghat et al.[25] who reported increased rates of *s2, s1a, s1as1bm2* in relation to severe chronic active gastritis. It is known that chronic active gastritis may proceed to gastric metaplasia, dysplasia that could progress into gastric cancer [32]. The subtype *vacAS1a,S1b* and was related mainly to sever gastritis, while the subtype *vacA, m2 and S1as1bm2* was related to moderate active gastritis. The association between *vacA s1/m1* and serious gastric epithelium damage has been previously documented [33]. Many studies revealed that *vacAs1, m1* rates between 24–84% [26].

In the current study no statistically significant differences were detected between *cagA* presence and endoscopic or histopathological reports, but more than 66% of duodenal ulcers had been related to positive *cagA*. Similar to these results, Seriki et al. [34] revealed that no relationship was found between the presence of *cagA* genes and clinical implications. Kadi et al. [14] revealed also no statistically significant association between *cagA* presence and histological abnormalities. Momenah et al.[23] revealed the presence of association between peptic ulcers and *cagA* (100%) and Marie et al.[11] reported correlation between peptic ulcers and *cagA* (71%).

In our study, a significant association was found between *iceA1* and *ice A2* genes presence with gastritis and gastric ulcer as *p* value was 0.027 and also there was correlation between *iceA1* and *iceA2* with chronic active gastritis, 40% and 45% respectively, while the association between *iceA* negative cases with chronic active gastritis was (15%) only. Several studies [14,23,24] agreed with association of *iceA1* presence with gastric ulcer and gastritis, while previous studies from Iran and Brazil revealed no relation between *iceA1* gene presence and gastritis [25, 35]

CONCLUSIONS

The current work reported an increased *H. pylori* prevalence rate which was about 67% in dyspeptic patients in Al Quwayiyah General Hospital, Saudi Arabia. There was a statistically significant association between *iceA1* and *iceA2* genotypes presence with gastric ulcer and gastritis. The identification of virulence factors of *H. pylori* allows the clinician to

identify high-risk patients associated with *H. pylori* infections.

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