Helicobacter pylori cagA and iceA genotypes status and risk of peptic ulcer in Saudi patients

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ABSTRACT

Objectives: To determine the prevalence of cagA+ and iceA genotypes among *Helicobacter pylori* (*H. pylori*) isolates from a group of Saudi patients with gastric complaints, and to find out any significant correlation between these strains and severe gastric clinical outcomes such as peptic ulcer and gastric cancer in Saudi population.

Methods: A total of 1104 gastric biopsies from 368 patients who presented with symptoms suggestive of chronic gastritis, peptic ulcer disease, or gastric carcinoma were taken from the main hospitals in the Western region of Saudi Arabia from July 2004 to July 2005. We cultured the samples for *H. pylori* and a polymerase chain reaction was carried out to check for the presence or absence of cagA gene and the status of iceA genotypes.

Results: Among the 368 suspected patients to be infected with *H. pylori* by means of clinical features and endoscopic findings; 103 (28%) were positive using culture technique. The relation of the presence of cagA and the development of cases to gastritis and ulcer was statistically significant (p=0.0001). Furthermore, this study revealed that 100% of ulcer cases were infected with iceA1 with a statistically significant correlation (p=0.0001), while 94.6% of gastritis and 90.9% of normal were infected with iceA2 (p=0.0001). Moreover cagA+/iceA1 combined genotypes was statistically correlated with peptic ulcer (100%) but not cagA-/iceA1 (0%; p=0.0001).

Conclusion: Certain *H. pylori* genotypes were more virulent than others. Multiple clinical implications based on these finding might be studied further.

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Helicobacter pylori (*H. pylori*) is a gram-negative curved rod with a tuft of 4-7 polar flagella. *Helicobacter pylori* induces gastric mucosal inflammation, which may progress into peptic ulcers.[1] Persistent infection also increases an individual's risk for development of gastric adenocarcinoma and gastric mucosa associated lymphoid tissue carcinoma.[2] In 1994, *H. pylori* was declared a group I carcinogen for gastric cancer.[3] Although gastritis is almost universal among infected individuals, some of patients develop peptic ulcer disease, and small fraction of these proceed in later life to develop gastric cancer.[4,5] One possibility to explain the variation in clinical outcome is that a considerable genetic variation exists between different strains of *H. pylori* and so some causing a considerable inflammatory response in the host than others. These strains are also associated with a greater likelihood of causing peptic ulcer, atrophic gastritis, intestinal metaplasia and gastric cancer. The major difference between virulent and relatively avirulent strains depends upon the presence of virulent genes in the virulent strains.[6] Two of the most virulence genes related to severe clinical outcomes are the cagA and iceA genes. Approximately 60-80% of *H. pylori* isolates in the world possess the cytotoxin-associated gene A (cagA), and its presence strongly correlated with expression of vacuolating cytotoxin activity.[7] Several recent studies have shown that cagA positive *H. pylori* strains are more likely associated with human infections that result in severe clinical outcomes such as ulcers or gastric cancer.[8-10] The iceA gene is a newly discovered gene. There are 2 main variants alleles of this gene: iceA1 and iceA2. The function of this gene is not yet fully known. Previous study reported a strong association between the presence of the iceA1 allele and peptic ulcer disease.[11] The aim of this study is to determine the prevalence of cagA+ and iceA genotypes among *H. pylori*

isolates from a group of Saudi patients with gastric complaints using PCR as a typing system and to find out a possible association between cagA status and iceA genotyopes and severe gastric clinical outcomes such as peptic ulcer and gastric cancer in Saudi patient.

Methods. A total of 1104 gastric biopsies from 368 patients were taken from antrum of the patients presenting the main hospitals in the Western region of Saudi Arabia. Average age of the patients was 16-90 years (mean of the age is 43 years). On examination, 284 (77.2%) were suffering from different gastric abnormalities being 264 (71.7%) with gastritis, 18 (4.9%) with gastric ulcer and 2 (0.5%) with tumor, while the remaining 84 (22.8%) were normal under endoscopic examination.

Gastric biopsies transported in a 0.5 ml Brucella broth media (Oxoid, UK). Three gastric biopsies were obtained from each patient, one was used for rapid CLO test (for determination of urease activity) (Oxoid, UK) and the others were cultured on *H. pylori* selective agar (Oxoid, UK) and incubated at 37°C in a BBL GasPak (Becton-Dickinson, USA) containing a Campy-Pak Plus microaerophilic system generator (Becton-Dickinson, USA) and incubated for 7 days. The identity of *H. pylori* clinical isolates were confirmed by colonial morphology, gram-stain (curved Gram-negative bacilli) and positive reaction for oxidase, catalase and urease tests.

Deoxyribonucleic acid was extracted and purified according to the method detailed by Pitcher et al.[12] Primers were designed to amplify 349 bp product within the cagA gene, 247bp within the iceA1 or 334 bp within iceA2 and 203 bp product within the Ure gene (positive control).[13-15] A 5ml of each PCR primer (0.025 µM final concentration) (TIB Molbiol, Germany) plus 5 ml of the extracted DNA were added to a PCR master mix (100 mM Tris-HCl, and 500 mM KCl at pH 8.3 at 20°C, 1.5 mM MgCl2, 200 µM each deoxyribonucleoside triphosphate, 0.025U Taq polymerase) (Qiagen, UK). This mixture was then heated to 94^0C for 5 minutes and then subjected to 35 cycles each of 95^0C for 1 minute, 52^0C for 1 minute, and 72^0C for 1 minute with a final extension of 72^0C for 7 minutes. A10 µl aliquot of each PCR product was loaded onto 1% agarose (Sigma, UK) stained with ethidium bromide (0.5 µg/ml; Sigma Ltd., USA) and run at 90V for approximately one hour prior to viewing under UVP BioDoct-It digital imaging system (UVP, Inc., Cambridge, UK).

Statistical analysis. Distribution of cagA among clinical isolates and its correlation with endoscopic findings were recorded and analyzed using SPSS (version 10) and Chi-squared test was used to compare frequencies using SPSS (version 10).

Results. Among the 368 suspected patients to be infected with *H. pylori* by means of clinical features and endoscopic findings 103 (28%) were positive using culture technique. Both malignant cases found to be *H. pylori* negative. The study showed that positive samples from gastritis, gastric ulcer, normal cases according to endoscopic findings were correlated with 70.8%, 6.6% and 22.6% with the presence of *H. pylori*. All the 103 cases were positive for Ure gene. Out of 103 cases, 54 cases (52%) were cagA+ (cagA positive). The distribution of iceA1 among the tested sample was 13 and iceA2 was 90. The results showed a high percentage of cagA (62.2%), and iceA2 (94.6%) in gastritis cases compared to gastric ulcer and normal cases (p=0.001). In case of gastric ulcer, the prevalence was 100% for both cagA+, and iceA1 (p=0.0001); while in normal cases, the highest prevalence rate was among iceA2 (90.9%) (p=0.001) (**Table 1**). The frequencies of cagA and iceA combined genotypes in relation to endoscopic findings are illustrated in **Table 2**. The frequency of cagA+/iceA2 combined genotypes was significantly higher in gastritis cases compared to gastric ulcer and normal cases (p=0.0001). On the other hand, the frequency of cagA+/iceA1 combined genotypes was significantly higher in gastric ulcer cases compared to gastritis and normal cases (p=0.0001). Moreover, cagA+/iceA1 combined genotypes was statistically correlated with peptic ulcer (100%) but not cagA-/iceA1 (0%; p=0.0001). Lastly,

Table 1 - Frequency and prevalence rate of cagA+, and iceA genotypes according to endoscopic findings.

Endoscopic findings	cagA	iceA	
		iceA1	iceA2
Gastritis	46 (62.2)	4 (5.4)	70 (94.6)
Ulcer	7 (100)	7 (100)	0 (0)
Normal	1 (4.5)	2 (9.1)	20 (90.9)

Data are expressed as number and (%).

Table 2 - The frequency and prevalence rate of cagA+ and iceA combined genotypes in relation to endoscopic findings.

Genotypes	Prevalence rate		
	Gastritis	Ulcer	Normal
cagA (+) / iceA1	4 (5.4)	7 (100)	0 (0)
cagA (+) / iceA2	42 (56.8)	0 (0)	1 (4.5)
cagA (-) / iceA1	0 (0)	0 (0)	2 (9.1)
cagA (-) / iceA2	28 (37.8)	0 (0)	19 (86.4)

Data are express as number and (%).
+ = positive; - = negative

the frequency of cagA-/iceA2 combined genotypes was significantly higher in normal cases compared to gastritis and gastric ulcer cases (p=0.0001).

Discussion. *Helicobacter pylori* is a spiral bacterium that inhabits the gastric mucosa of the human stomach in approximately half of the world's population for a life time.[1,16] Infection of this unique ecological niche by *H. pylori* induces gastric mucosal inflammation, which may progress into peptic ulcer. Persistent infection also increases an individual's risk for development of gastric adenocarcinoma and gastric MALToma.[17] Recent studies have shown that different cagA and iceA *H. pylori* genotypes can lead to different clinical outcome consequences in certain populations.[6,17] The prevalence of *H. pylori* in our study was 28% out of the total patients tested. This finding is much less than what has been reported elsewhere in the Kingdom with rates including 87% in the Eastern region, 61.6% in Central and Western regions.[18] This variability in the rates among different studies including this study may be attributable to the differences in the methods of identification in different studies, different demographic distribution of the bacteria among various regions, and previous antibiotic consumption.[19] These reasons may also explain the different distribution of this organism among different international studies.[19-21] The prevalence of cagA positive *H. pylori* isolates varies from one geographic region to another, such as 38% in Chile, 81% in the United Sates, 97% in Korea and 93% in Nigeria.[22-26] In this study, the prevalence of cagA was 52.4% among patients tested using PCR. The distribution of cagA was 62.2% in gastritis, 100% in gastric ulcer and 4.5% in normal cases. The relationship of the presence of cagA and the development of gastritis and gastric ulcer is statistically significant (p=0.0001), which further substantiate the role of cagA as a marker for increased virulence of *H. pylori*. These findings are in agreement with several previous studies.[27,28] In this study, analysis of iceA genotypes demonstrated that 87.4% of the positive *H. pylori*cases were iceA2 positive compared to only 12.6% cases positive for iceA1. IceA1 expression is associated with a higher activity of the gastric inflammation, a condition that increases the risk for developing ulcer disease and gastric carcinoma.[29] This study revealed that 100% of ulcer cases were infected with iceA1 with a statistically significant correlation (p=0.0001), while 94.6% of gastritis and 90.9% of normal were infected with iceA2. Previous studies in the United State and the Netherlands have demonstrated a strong association between iceA1 and ulcer disease, which also proved by this study.[13,30,31]

In conclusion the finding of this study might be used for identification of high-risk patients who are infected by more pathogenic *H. pylori* strains. Eventually, patients infected with such strains could be selected for prophylactic anti-Helicobacter treatment to prevent peptic ulcer disease and gastric carcinoma later on in their life.

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