brief report

The distribution of *cagA* and *dupA* genes in *Helicobacter pylori* strains in Kurdistan region, northern Iraq

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BACKGROUND AND OBJECTIVES: *Helicobacter pylori* is a Gram negative bacteria that causes peptic ulceration and gastric adenocarcinoma. *H pylori* virulence factors, such as *cagA* and *dupA*, are important to study in populations as they contribute to disease risk. This study aimed to look at the distribution of the *cagA* and *dupA* genes in *H pylori* strains isolated from patients suffering from gastroduodenal diseases in Kurdistan region, Iraq.
DESIGN AND SETTINGS: A cross-sectional study conducted between June 2011 and January 2012. Biopsies were collected from the Endoscopy Department in Duhok and Sulaimania hospitals, Kurdistan region, northern Iraq.
PATIENTS AND METHODS: Upper gastrointestinal (GI) endoscopy examination was performed and 4 gastric biopsies (2 from the antrum and 2 from the corpus) were obtained from 204 patients. *H pylori* positivity was examined by CLO test; then the association between disease status and virulence factors was assessed by polymerase chain reaction.
RESULTS: 154 (75%) of our samples were found to be *H pylori* + by CLO test. Endoscopic diagnoses for those who were positive were as follwos: peptic ulcer disease (PUD) including duodenal ulcer, 45; gastric ulcer, 23; and no ulcer (NPUD), 86. The overall prevalence rates of *cagA* and *dupA* were 72.7% and 18.8%, respectively. While a significant association between *cagA* and PUD was observed ($P \leq .017$; OR=0.4; CI=0.18–0.85), no relationship between *dupA* and PUD could be seen.
CONCLUSION: These data suggested that the presence of *cagA* may be a predictor of clinical outcome in Kurdistan region, northern Iraq.

Helicobacter pylori is a clinically important pathogen that colonises about 50% of the world's population.[1] *H pylori* is a Gram negative spiral-shaped organism, which prefers to live in microaerophilic conditions. It can chronically reside in the harsh environment of the stomach and causes subclinical gastritis in the majority of patients. However, infection with this bacterium may cause peptic ulcer disease (PUD). It has been found that 75% of gastric ulcers (GUs) and 90% of duodenal ulcers (DUs) are believed to be due to infection with this bacteria.1 Additionally, *H pylori* infection can predispose to the development of gastric cancer: adenocarcinoma and mucosa-associated lymphoid tissue lymphoma.[2,3]

The cytotoxin-associated gene A (*cagA*) is a part of the cag pathogenicity island (PAI). PAI is composed of genes that decode type IV secretion system, and this secretion system is responsible for the translocation of *CagA* into host cells.[4] Worldwide, 60% to 80% of *H pylori* strains possess *cagA*. The presence of *cagA* is correlated with PUD and gastric cancer.[5,6]

Another *H pylori* virulence factor, a homologue of virB4, was identified recently.[7] The initial report described a significant association between its presence and DU in various populations, hence the name DU promoting gene A (*dupA*).[7] Subsequent reports have confirmed the association of *H pylori* with DU in northern India,[8] and have shown a significant asso-

ciation with gastric adenocarcinoma in Belgium, South Africa, China, and the USA.[9] The aim of this paper was to study the distribution of *cagA* and *dupA* genes in Iraqi *H pylori* strains and their relationship with disease status.

PATIENTS AND METHODS

Clinical samples

All patients studied were referred to the Endoscopy Department in Duhok and Sulaimania hospitals, Kurdistan region, northern Iraq. Upper gastrointestinal (GI) endoscopy examination was performed and 4 gastric biopsies (2 from the antrum and 2 from the corpus) were obtained from 204 patients. Endoscopic diagnoses were as follows: PUD, 70 and NPUD, 134.

Rapid urease test

One antral and 1 corpus biopsy were inoculated into CLO gel. The results were observed and recorded within 24 hours. A positive CLO was indicated when the color changed from yellow to pink.

DNA extraction

DNA was extracted directly from the biopsy specimens and used for polymerase chain reaction (PCR)-based *H pylori* typing. DNeasy tissue kits (QIAGEN, Germany) were used to extract DNA from biopsies following the manufacturer instructions.

Genotyping of **H pylori**

Thermal cycling for amplifying *cagA* was 95°C for 30 seconds, 50°C for 1 minute, and 72°C for 2 minutes, for a total of 35 cycles. PCR amplification of *cagA* used previously described primers cag2 (5'-GGAACCCTAGTCGGTAATG-3') and cag4 (5'-ATCTTTGAGCTTGTCTATCG-3') to amplify about 500-base pair (bp) product from the middle of *cagA* (PAI).[10] The *dupA* was amplified by using standard protocols with the following block cycler conditions: 35 cycles, each consisting of denaturation at 95C for 30 seconds, annealing at 50°C for 1 minute, and elongation at 72°C for 2 minutes. Two primers were used: *DupA*F113 (5'-GACGATTGAGCGATGGGAATAT-3') and *DupA*R1083(5'-CTGAGAAGCCTTATTATCTT GTTGG-3').[11] Amplification of these genes started with an initial denaturation at 95°C for 60 seconds and a final elongation step of 5 minutes at 72°C. Reactions were performed in 25 µL volume containing 1 µL of *H pylori* genomic DNA, 1 µL of primer, 0.5 µL of Taq DNA polymerase, 0.5 µL of dNTP, and 2.5 10× PCR buffer. Then 5 µL of the PCR products were electrophoresed in 1.5% agarose gels for 40 minutes at 80 V in 1× TAE buffer. All gels were stained with ethidium bromide (1 mg/L) and photographed under UV light. A 100-bp DNA ladder (Gibco, Paisley, UK) was used as a size marker (M) in all gels. For *cagA*, and *dupA*, 8823 and J99 strains were used as a positive control, respectively.

The study protocol was approved by the Ethics and Research Committees of the individual hospitals, and all patients were given informed consent to the study.

Data analysis

The statistical analysis of data was performed by using chi-square test with significance set at a *P* value of <.05 using Minitab 15 software (Minitab Inc, Pennsylvania, USA).

RESULTS

The mean age (standard deviation) of our patients was 36 (17) years. A total of 154 (75%) of our samples were found to be *H pylori*+ by CLO test. Endoscopic diagnoses for those who were positive were as follows: PUD including DU, 45; GU, 23; and no ulcer (NPUD), 86. No cancer was observed.

The *cagA* gene was observed in 112 (72.7%) isolates (**Table 1, Figure 1**). Among our samples, 56/68 (82%) peptic ulcer patients carried *cagA*+ strains, significantly more than the 56/86 (65%) non-ulcer patients ($P \leq .017$; OR=0.4; CI=0.18–0.85). Considering duodenal and GU separately in this population, 21 (91%) patients with GU had *cagA*+ strains, compared with 56 (65%) with no ulcer ($P<.014$; OR=0.18; CI=0.03–0.8). A total of 35 (78%) Iraqi DU patients had *cagA*+ strains (*P*=not significant [ns] compared with no ulcer).

The overall prevalence of the *dupA*+ genotype was 18.8% (29/154 isolates) (**Figure 2**). The difference in the prevalence of *dupA* positivity was not significant between patients with NUD (16%) and PUD (28%) (*P*=ns) (**Table 2**).

DISCUSSION

The prevalence of *H pylori* infection ranged from more than 70% in developing countries such as Bangladesh, India, and Mexico to around 20% in developed countries such as Netherland and Australia.[12] It was previously found that inadequate sanitation practices, low social class, and crowded or high-density living conditions seem to be related to a higher prevalence of *H pylori* infection.[13] Hence, this high prevalence of *H pylori* infection in our population may be due to poor hygiene and crowded conditions.

CagA protein, which is encoded by *cagA* gene with-

Table 1. *cagA* status among *H pylori* strains from unselected Iraqi patients with dyspepsia. A significant association was found between gastric ulcer and *cagA*.

	GU	DU	PUD (DU and GU)	NPUD
cagA+ (n)	21	35	56	56
cagA– (n)	2	10	12	30
Total	23	45	68	86

GU: Gastric ulcer, DU: duodenal ulcer, PUD: peptic ulcer disease, NPUD: no peptic ulcer disease.

Table 2. *dupA* status among *H pylori* strains from unselected Iraqi patients with dyspepsia. No association was found between clinical outcome and *dupA, cagA*, cytotoxin-associated A gene.

	GU	DU	PUD (DU and GU)	NPUD
dupA+ (n)	4	11	15	14
dupA– (n)	19	34	53	72
Total	23	45	68	86

GU: Gastric ulcer, DU: duodenal ulcer, PUD: peptic ulcer disease, NPUD: no peptic ulcer disease, *dupA*: duodenal ulcer promoting A gene.

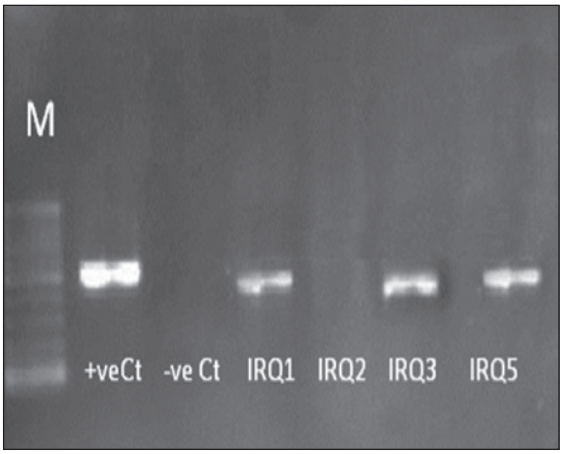

Figure 1. Characterization of Iraqi strains for *cagA* gene. Two per cent gel electrophoresis of *H pylori* genotypes showing polymerase chain reaction results of cagA gene. Lane M is a 100-bp ladder. IRQ1, IRQ3, and IRQ4 typed as *cagA+*, while IRQ3 typed as *cagA*–. Negative Ct is negative control and positive Ct is positive control.

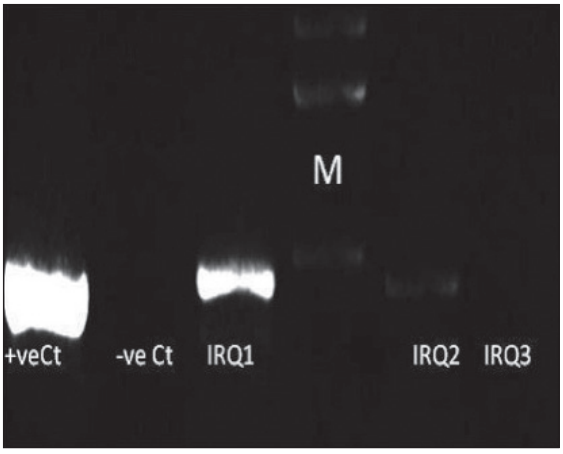

Figure 2. Characterisation of Iraqi strains for *dupA* gene. Two per cent gel electrophoresis of *H pylori* genotypes showing polymerase chain reaction results of *dupA* gene. Lane M is a 100-bp ladder. IRQ1 and IRQ2 typed as *dupA+*, while IRQ3 typed as *dupA*–. Negative Ct is negative control and positive Ct is positive control.

in the cag PAI, is produced by the vast majority of *H pylori* strains. The *cagA* has been found to associate significantly with an increased risk for the development of atrophic gastritis, PUD, and gastric cancer.[5,6] We looked within our population for associations between virulence factors and PUD. Among our strains, we observed an association between *cagA*+ status and PUD. Reports from neighboring countries, Turkey and Saudi, have shown similar results to ours.[14-16] However, conflicting results have been reported in Iran, probably due to the difference between *cagA* prevalence among Iranian regions.[14,17-19] Additionally, we showed that *cagA* is not associated with DU, which may be a type II error due to the sample size. More research is needed to investigate the role of *H pylori* virulence factors and their role in upper GI diseases.

In a study conducted using samples from South Korea, Japan, and Colombia, it was shown that the presence of *dupA* was significantly associated with DU and negatively associated with gastric cancer. In the same study, *dupA* appeared to increase interleukin-8 secretion from gastric mucosa.[7] Additionally, this correlation between *dupA* and DU was shown in a population from northern India.[8] However, no significant association between *dupA* prevalence and ulceration or cancer was found in populations from Brazil.[20] Alternatively, it was found that *dupA* was significantly associated with gastric cancer development in populations from Belgium, South Africa, China, and the USA, but was not significantly associated with DU.[9] Previously, it was also shown that the presence of *dupA* was associated with more intense antral neutrophil infiltration in populations from East Asia and South America[7] but not from Iraq.[21] In this project, no association was found between *dupA* and PUD. Again, this may be due to the sample size or due to the presence of polymorphisms in the *dupA*, such as the one described by Hussein et al.[11] These differences may be similar to the differences found in vacA and *cagA* phosphorylation motifs.

In conclusion, while a significant association between *cagA* and PUD was observed, no relationship between

dupA and PUD was seen. Studies from different parts of Iraq including different ethnic groups are needed to reach solid conclusions about the relationships between *H pylori* virulence factors and clinical outcomes.

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