brief report

The distribution of \textit{cagA} and \textit{dupA} genes in \textit{Helicobacter pylori} strains in Kurdistan region, northern Iraq

Azad M. Salih,\textsuperscript{a} Amer Goreal,\textsuperscript{a} Nafwaf R. Hussein,\textsuperscript{a} Shahla M. Abdullah,\textsuperscript{b} Khidir Hawrami,\textsuperscript{b} Mahde Assafi\textsuperscript{c}

From the \textsuperscript{a}School of Medical Sciences, University of Duhok, Duhok, Iraq; \textsuperscript{b}Genomic Centre, Koya University, Koya, Kurdistan Region, Iraq; \textsuperscript{c}CBS University of Nottingham, Nottingham, United Kingdom

Correspondence: Dr. Nafwaf R. Hussein · School of Medical Science, University of Duhok, Iraq · T: +964 (0)62 762 0004, F: +964 (0)62 722 4401 · nafwaf.hussein@yahoo.com

Ann Saudi Med 2013; 33(3): 290-293
DOI: 10.5144/0256-4947.2013.290

BACKGROUND AND OBJECTIVES: \textit{Helicobacter pylori} is a Gram negative bacteria that causes peptic ulceration and gastric adenocarcinoma. \textit{H pylori} virulence factors, such as \textit{cagA} and \textit{dupA}, are important to study in populations as they contribute to disease risk. This study aimed to look at the distribution of the \textit{cagA} and \textit{dupA} genes in \textit{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. \textit{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 \textit{H pylori} + by CLO test. Endoscopic diagnoses for those who were positive were as follows: peptic ulcer disease (PUD) including duodenal ulcer, 45; gastric ulcer, 23; and no ulcer (NPUD), 86. The overall prevalence rates of \textit{cagA} and \textit{dupA} were 72.7\% and 18.8\%, respectively. While a significant association between \textit{cagA} and PUD was observed (\(P \leq 0.017\); OR=0.4; CI=0.18–0.85), no relationship between \textit{dupA} and PUD could be seen.

CONCLUSION: These data suggested that the presence of \textit{cagA} may be a predictor of clinical outcome in Kurdistan region, northern Iraq.

\textit{Helicobacter pylori} is a clinically important pathogen that colonises about 50\% of the world’s population.\textsuperscript{1} \textit{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.\textsuperscript{1} Additionally, \textit{H pylori} infection can predispose to the development of gastric cancer: adenocarcinoma and mucosa-associated lymphoid tissue lymphoma.\textsuperscript{2,3} The cytotoxin-associated gene A (\textit{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 \textit{CagA} into host cells.\textsuperscript{5} Worldwide, 60\% to 80\% of \textit{H pylori} strains possess \textit{cagA}. The presence of \textit{cagA} is correlated with PUD and gastric cancer.\textsuperscript{5,6}

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

**cagA AND dupA GENES IN H pylori**

mination with gastric adenocarcinoma in Belgium, South Africa, China, and the USA. 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 DNA extraction. DNA was extracted directly from the biopsy specimens and used for polymerase chain reaction (PCR)-based DNA extraction. The color changed from yellow to pink.

**Data analysis**

The statistical analysis of data was performed by using chi-square test with significance set at a *P* value of <0.05 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, 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* ≤.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=0.03–0.38). 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. 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. 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-
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 America7 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

---

**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*+  | 21 | 35 | 56              | 56   |
| *cagA*−  | 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*+  | 4  | 11 | 15              | 14   |
| *dupA*−  | 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.
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.

REFERENCES

1. Atherton J. The Pathogenesis of H pylori–induced Gastro-Duodenal Diseases. Annual Review of: Mechanisms of Disease 2006;1:63-96.
2. Bandipalliam P. MALT lymphomas—a closer look in the genomics era. South Med J 2006;99(12):1322-4.
3. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitia F. Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. Brmj 1991;302(6788):1302-5.
4. Akopyants NS, Clifton SW, Kersulyte D, Crabtree JE, Youree BE, Reece CA, Bukarov NO, Drazeck ES, Roe BA, Berg DE. Analyses of the cag pathogenicity island of Helicobacter pylori. Mol Microbiol 1996;28(1):37-53.
5. Atherton JC. H pylori virulence factors. Br Med Bull 1996;54(1):105-120.
6. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peak RM, Chyou PH, Stemmermann GN, Nomura A. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995;55(10):2311-5.
7. Lu H, Hsu PI, Graham DY, Yamaoka Y. Duodenal ulcer promoting gene of Helicobacter pylori. Gastroenterology 2005;128(1):833-848.
8. Arachchi HS, Kalra V, Lal B, Bhatia V, Baba CS, Chakravarthy S, Rohatgi S, Sarma PM, Mishra V, Das B, Ahuja V. Prevalence of duodenal ulcer-promoting gene (dupA) of Helicobacter pylori in patients with duodenal ulcer in North Indian population. Helicobacter 2007;12(6):591-7.
9. Argent RH, Burette A, Mienjie Deyi VY, Atherton JC. The presence of dupA in Helicobacter pylori is not significantly associated with duodenal ulceration in Belgium, South Africa, China, or North America. Clin Infect Dis 2007;45(9):1204-6.
10. Rudi J, Kolb C, Maivald M, Kuck D, Sieg A, Stremmel W. Diversity of Helicobacter pylori vacA and cagA genes and relationship to VacA and CagA protein expression, cytotoxin production, and associated diseases. J Clin Microbiol 1996;36(4):944-948.
11. Hussein NR, Argent RH, Marx CK, Patel SR, Robinson K, Atherton JC. Helicobacter pylori dupA Is Polymorphic, and Its Active Form Induces Proinflammatory Cytokine Secretion by Mononuclear Cells. Journal of Infectious Diseases 2010;202(2):261-269.
12. Khalifa M, Sharaf R, Aziz R. Helicobacter pylori: a poor man's gut pathogen? Gut Pathogens 2010;2(1):2.
13. Brown LM. Helicobacter Pylori: epidemiology and Routes of Transmission. Epidemiologic Reviews 2000;22(2):283-297.
14. Hussein NR, Mohammadi M, Talebkhani Y, Mohagheghi M, Vaziri H, Eshagh Hosseini M, Mohajerani N, Dghali A, Zanninia L, cagA Gene and Protein Status Among Iranian Helicobacter pylori Strains. Digestive Diseases and Sciences 2008;53(3):925-932.
15. Talebkhani Y, Mohammadi M, Mohagheghi M, Vaziri H, Eshagh Hosseini M, Mohajerani N, Dghali A, Zanninia L. cagA and iceA genotypes status and risk of 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.