Detection and Genotyping of Helicobacter pylori among Gastric ulcer and Cancer Patients from Saudi Arabia

Fehmida Bibi1, Sana Akhtar Alvi2, Sara Ali Sawan3, Muhammad Yasir4, Ali Sawan5, Asif A. Jiman-Fatani6, Esam I. Azhar7

ABSTRACT

Background and Objectives: Helicobacter pylori (H. pylori) infection is cause of several gastrointestinal diseases in humans. Virulence genes of H. pylori are associated with severity of disease and vary geographically. The aim of present study was to detect H. pylori in formalin-fixed paraffin-embedded (FFPE) tissues and further investigate prevalence of babA2, cagA, iceA1, iceA2, vacA s1/s2 and vacA m1/m2 genotypes in H. pylori from gastric cancer (GC) and gastric ulcer (GU) patients' biopsy samples.

Methods: We used FFPE tissues of 35 GC and 10 GU patients' biopsy samples. Using Polymerase Chain Reaction (PCR), detection of H. pylori strain was performed by using specific primers targeting 16S rRNA and ureC encodes for phosphoglucomutase genes. We have identified different virulence genes of H. pylori by PCR.

Results: Of all the 45 samples tested, 20 GC and all 10 GU samples were positive for identification of H. pylori using specific genes (16S rRNA and ureC). The prevalence of babA2 (100%) was significantly higher in GC as compared to GU (40%) samples. The rate of virulence genes vacAs1 was higher in both GU (80%) and GC (100%).

Conclusions: Our study finds that vacAs1am1 and babA2 are most prominent genotypes and may play role in development of Gastric cancer.

KEY WORDS: H. pylori, Genotyping, Gastric ulcer, Gastric cancer.

doi: https://doi.org/10.12669/pjms.332.12024

How to cite this:
Bibi F, Alvi SA, Sawan SA, Yasir M, Sawan A, Jiman-Fatani AA, et al. Detection and Genotyping of Helicobacter pylori among Gastric ulcer and Cancer Patients from Saudi Arabia. Pak J Med Sci. 2017;33(2):320-324.
doi: https://doi.org/10.12669/pjms.332.12024

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Helicobacter pylori (H. pylori) is a pathogenic bacterium that inhabits gastric mucosa of humans and cause several gastrointestinal diseases including gastric cancer (GC). In developing countries prevalence of H. pylori infection may exceed as compared to developed countries where 20-50% are affected with infection.1 Previous studies suggest that H. pylori is genetically variable and certain genotypes are only detected in certain populations.2 Several virulence factors of H. pylori strain, such as babA2, cagA, vacA, and iceA1 have been identified and involved in pathogenesis of infection.3 Recently reported blood group antigen-binding adhesin (BabA), a membrane protein of H. pylori help in binding to gastric epithelium. Three different bab alleles have been reported where only the babA2 is functional for binding activity.4 Previous studies have reported prevalence of babA2 positive strain in peptic ulcer and GC but with conflicting results.5 Therefore, relationship between H. pylori genotype and disease condition may vary from one geographic region to other.
The cytotoxicity-associated gene A (cagA) is present in almost 50% of H. pylori strains and is constituent of genomic pathogenicity island (cag-PAI) responsible for type IV secretion system. The cagA-positive strains of H. pylori are responsible for mucosal inflammation and interleukin-8 (IL-8) production and are associated with pathogenesis of gastric cancer. In Asian countries, rate of cagA positivity has been reported in almost all strains of H. pylori isolated from infected cases.7

The vacuolating cytotoxin gene (vacA) encodes for vacuolating cytotoxin and found in all strains of H. pylori. It is involved in pathogenesis of peptic ulcer and GC by injuring gastric epithelial cells.8 All strains of H. pylori contain the vacA gene, and have two variable parts i.e signal region (s1/s2) and middle region (m1/m2). Previous studies have reported vacA allelic variations in different geographical regions as well as their toxic activity.9

Recently another virulence gene of H. pylori, iceA (the induced by contact with epithelium) has been reported. The iceA gene has two allelic types, iceA1 and iceA2 where iceA1 is significantly expressed and associated with peptic ulceration. The ureC gene encodes for phosphoglucomerase mutase, and renamed as glmM gene. The ureC (glmM) gene is easily detectable in H. pylori strains and involved in the development of the bacterial cell wall and growth.10

In Saudi Arabia, H. pylori infection rate is high in the Eastern, Central and Western region of the Kingdom. Previous studies have reported some virulence genes such as cagA, iceA1, and iceA2 in peptic ulcer and gastric ulcer in Saudi population. Detection of virulence genes of H. pylori mentioned above has not been reported yet in GC patients in Saudi Arabia. The genotyping of H. pylori is useful to determine epidemiological importance of H. pylori strain. Therefore, we designed a study to detect the presence of H. pylori using specific glmM and 16S rRNA. Further prevalence of virulence genes babA1, cagA, iceA1, iceA2, vacA s1/s2 and vacA m1/m2 from paraffin-embedded (FFPE) gastric biopsies collected from GC and GU Saudi patients have been studied.

METHODS

Biopsy samples Collection and DNA extraction: Gastric FFPE biopsy specimens were collected from 35 GC patients and 10 with GU who had undergone gastric endoscopy in King Abdulaziz University (KAU) hospital in Jeddah, Saudi Arabia, between 2000-2014. Written informed consent was taken from all the patients. This study was approved by the Ethics and Research Committees of the hospital KAU. DNA was extracted from the biopsies using QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). Extracted DNA was further used for PCR.

Polymerase Chain Reaction (PCR) assays: To detect H. pylori strain in biopsy samples from GC and GU patients specific primers targeting 16S rRNA and ureA genes were used. Further prevalence of virulence factors of H. pylori strain, specific primers targeting babA1, cagA, iceA1 iceA2, vacA s1/s2, vacA m1/m2 were used for PCR (Table-I). Amplification

| Genes          | Nucleotide sequence (5′-3′)                        | PCR product (bp) | PCR conditions          | Reference |
|---------------|---------------------------------------------------|------------------|-------------------------|-----------|
| 16S rRNA      | GCGGACCTGCCTGGAACATTAC                           | 138 bp           | 95°C, 50 s; 60°C, 50 s; | 28        |
|               | CTGTTAGCTGATTACCTGGAGA                           |                  | 72°C, 50 s (35 cycles)  |           |
| glmM          | AAGCCTTATTAGGGGTAGGGGT TT                       | 294 bp           | 95°C, 50 s; 56°C, 50 s; | 23        |
|               | AAAGCCTTTTCATACACGCTAAGGC                        |                  | 72°C, 50 s (35 cycles)  |           |
| babA2         | CCAAAAGCCAAACAAAAAGACGT                         | 271 bp           | 95°C, 45 s; 50 s;       | 29        |
|               | GCTGTTGTAAGAAAGCCTGT                            |                  | 72°C, 50 s (35 cycles)  |           |
| iceA1         | GGTGTTTTTACAACAAAATGTC                           | 247 bp           | 95°C, 50 s; 50°C,       | 29        |
|               | CTTAGGCCATTACCTTTTGA                             |                  | 72°C, 50 s (35 cycles)  |           |
| iceA2         | GTGTTAGTATCATACAAATTAT                          | 229 bp           | 95°C, 50 s; 55°C,       | 28        |
|               | TTTCCCTATTTCTAGGAT                              |                  | 72°C, 50 s (35 cycles)  |           |
| cagA          | TTAGCCAAACACAACAAAAAGCACG                      | 183 bp           | 95°C, 50 s; 60°C,       | 35        |
|               | CTCTCCCTATTTGCAGAT                              |                  | 72°C, 50 s (35 cycles)  |           |
| vacA s1/s2    | AICGGGAATACAACAAAAACACAC                        | 259/286 bp       | 95°C, 50 s; 58°C,       | 20        |
|               | CAGCCTTGAATGCCCAAC                               |                  | 72°C, 50 s (35 cycles)  |           |
| vacA m1/m2    | CAATCTGTCCAATCAAGCGGAG                         | 567/642 bp       | 95°C, 50 s; 54°C,       | 14        |
|               | GCCCTAAAATAATTCAAGG                             |                  | 72°C, 50 s (35 cycles)  |           |
was conducted in a total volume of 25µL. The reaction mixture contained 13µL, 2X ready PCR mix (Thermo Scientific), 2µL of each forward and reverse primers (Table-I), 1 µg DNA template, and 9µL nuclease free distilled water to a total volume of 25µL. The PCR amplification was performed according to the following program: an initial denaturation at 95°C for five minutes, followed by 35 cycles of denaturation at 95°C for 50s, annealing for 50s (Table-I), and a final extension at 72°C for five minutes. The amplified PCR products were electrophoresed (100 V/35 min) using 1% agarose gel. DNA ladder of 100 bp (Norgen, Canada) was used to determine the size of the amplified bands.

**Statistical Analysis:** Data of two groups were compared by using chi-square test using SPSS statistical software Version 9 (SPSS Inc., Chicago, IL, USA). A P value less <0.05 was considered as significant.

**RESULTS**

This study included 45 FFPE gastric biopsy samples from Saudi patients. A total of 45 gastric patients, 13 (28.8%) females and 32 (71.2%) males ranging in age from 18 to 87 years, were included in the study.

Among 45 cases, 35 (77.7%) were diagnosed as GC and 10 (22.3%) were GU cases (Table-II).

The detection of *H. pylori* was investigated using PCR. Among 45 FFPE gastric tissues, 20 (57.2%) GC and 10 (100%) GU biopsy samples were positive for *H. pylori* using 16S rRNA (Fig. 1a and b) and glmM genes. We examined six different *H. pylori* virulence genes in gastric biopsy samples. Among *H. pylori* positive gastric tissues prevalence of *babA2* gene was higher (100%) in GC tissues as compare to GU (4%). The presence of *cagA* gene yield a fragment of 183 bp using PCR. Amplification of *iceA1* and *iceA2* gene was performed using specific primers (Table-II). Prevalence of both *iceA1* and *iceA2* was detected only in three GC samples (15%) and was negative for GU samples. Only eight samples (40%) from GC and three (30%) from GU was positive for *cagA*. There was no significant difference for different genotypes among two different goups of patients (P > 0.05).

The detection of *vacA s1/s2* allele showed presence in 20 (100%) samples from GC and 8 (80%) from GU. The most virulent *vacA s1* was detected in most of the samples as it was detected in 12 (60%) samples from GC and 5 (50%) samples from GU. Whereas, *vacA s2* was not detected in any sample. Combined *vacA s1/s2* was detected in 8 (40%) GC and three (30%) GU samples. Detection of *vacA m1/m2* yielded fragment of 567/642 bp product. Only two (20%) samples were positive for *vacA m2* from GU samples. While for GC samples, allele *vacA m1* was detected in 3 (15%) samples and *vacA m2* was also detected in 3 (15%) samples. Combined *vacA m1/m2* was not detected in any sample (P > 0.05).

**Table-II: Demographic characteristics and prevalence of virulence genes in different groups.**

|                | Group I (GU) (n=10) | Group II (GC) (n=35) |
|----------------|--------------------|----------------------|
| Age: mean      | 31.3±7.5           | 58.2±14.6            |
| Gender:        |                    |                      |
| Male           | 3 (30%)            | 29 (82.9%)           |
| Female         | 7 (70%)            | 6 (17.1%)            |
| babA2          | 4 (4%)             | 20 (100%)            |
| iceA1          | 0 (0)              | 3 (15%)              |
| iceA2          | 0 (0)              | 3 (15%)              |
| cagA           | 3 (30%)            | 8 (40%)              |
| vacA s1        | 5 (50%)            | 12 (60%)             |
| vacA s2        | 0 (0)              | 0 (0)                |
| vacA s1 s2     | 3 (30%)            | 8 (40%)              |
| vacA m1        | 0 (0)              | 3 (15%)              |
| vacA m2        | 2 (20%)            | 3 (15%)              |
| vacA m1/m2     | 0 (0)              | 0 (0)                |
In our study vacA s1 was common allele in GC while vacA s1/s2 was predominant genotype in GU samples (Table-II).

**DISCUSSION**

Gastric cancer is an important cause of the death not only in Saudi Arabia but all over the world. Several previous studies have reported the importance of the *H. pylori* virulence genotypes. However, very few studies are available regarding *H. pylori* genotypes in Saudi population. Our study has confirmed high prevalence of *H. pylori* infection in GC and GU biopsy samples. In this study we have investigated the prevalence of various virulence factors (babA2, cagA, iceA1, iceA2, vacA s1/s2, vacA m1/m2) from *H. pylori* positive GC and GU biopsy samples using PCR. Prevalence of *H. pylori* and Gastric Cancer is high in Asian countries such as Japan and Korea as compared to other countries. In this study we have seen high prevalence of *H. pylori* virulence factors in GC and GU samples. Different genes contribute in colonization of *H. pylori* to gastric epithelium of humans. The glmM and 16S rRNA gene has more sensitivity than other genes for detection in gastric biopsies samples. Therefore, we have used glmM and 16S rRNA for detection of *H. pylori* in biopsy samples.

Virulence genes of *H. pylori* cagA and vacA are important adherence factors involved in gastrointestinal diseases. In our study we found low frequency of cagA in both groups. cagA is associated with the development of gastric carcinoma and it is an important marker for the most virulent strains associated with gastric severe infection. Previous studies have shown relationship of gastric cancer and cagA positive *H. pylori* while other show contradictory results like we have in our study. We have used VacAs1/s2 and VacAm1/m2 type virulence factor for detection and according to our results prevalence of VacAs1 is high in both GU and GC. VacAs1 genotype is associated with high toxin activity and severe diseases. Previous study from China has also reported similar results.

The babA2 gene has been shown to be associated with high risk of gastric cancer and also has strong relation with VacAs1. In GC samples we have found high prevalence of babA, and VacAs1. A previous study has reported similar results where these genotypes were associated with increased risk of developing cancer.

**REFERENCES**

1. Miwa H, Go MF, Sato N. *H. pylori* and gastric cancer: the Asian enigma. Am J Gastroenterol. 2002;97(5):1106–1112. doi: 10.1111/j.1572-0241.2002.00563.x
2. Sincischi LA, Corea P, Pek RM, Camargo MC, Delgado A, Piazuelo MB, et al. *Helicobacter pylori* genotyping and sequencing using paraffin-embedded biopsies from residents of Colombian areas with contrasting gastric cancer risks. Helicobacter. 2008;13(2):135–145. doi: 10.1111/j.1523-5378.2008.00554.x
3. Erzin Y, Koksal V, Altun S, Dobrucali A, Aslan M, Erdamar S, et al. Prevalence of *Helicobacter pylori* vacA, cagA, cagE, iceA, babA2 genotypes and correlation with clinical outcome in Turkish patients with dyspepsia. Helicobacter. 2006;11(6):574–580. doi: 10.1111/j.1523-5378.2006.00461.x
4. Pride DT, Meinersmann RJ, Blaser MJ. Allelic variation within *Helicobacter pylori* babA and babB. Infect. Immun. 2001;69(2):1160–1171. doi: 10.1128/IAI.69.2.1160-1171
3. Chomvarin C, Namwat W, Chaicumpar K, Mairiang F, Angchan A, Ripa B, et al. Prevalence of Helicobacter pylori vacA, cagA, cagE, iceA and babA2 genotypes in Thai dyspeptic patients. Int J Infect Dis. 2008;12(1):30–36. doi: 10.1016/j.ijid.2007.03.012

5. Al Qabandi A, Mustsfa AS, Siddique I, Khajah AK, Madaa JP, Junaid TA. Distribution of vacA and cagA genotypes of Helicobacter pylori in Kuwait. Acta Trop. 2005;93(3):283-288. doi: 10.1016/j.actatropica.2005.01.004

10. Peek Jr RM, Thompson SA, Donahue JP, Tham KT, Atherton JC, Blaser MJ, et al. Adherence to gastric epithelial cells induces expression of a Helicobacter pylori gene, iceA, that is associated with clinical outcome. Proc Assoc Am Physicians. 1998;110(6):531–544.

10. Mattar R, dos Santos AF, Eisig JN, Rodrigues TN, Silva FM, Lupinacci RM, et al. No correlation of babA2 with vacA and cagA genotypes of Helicobacter pylori and grading of gastritis from peptic ulcer disease patients in Brazil. Helicobacter. 2005;10(6):601–608. doi: 10.1111/j.1523-5578.2005.00360.x

18. Miehlike S, Schuppler M, Frings C, et al. Helicobacter pylori vacA, iceA and cagA status and pattern of gastritis in patients with malignant and benign gastroduodenal disease. Am J Gastroenterol. 2001;96(4):1008–1030. doi: 10.1111/j.1572-0241.2001.03685.x

26. Smith SI, Kirsch C, Oyedeji KS, Arigbabu AO, Coker AO, Bayerdöffer E, et al. Prevalence of Helicobacter pylori vacA, cagA and iceA genotypes in Nigerian patients with duodenal ulcer disease. J Med Microbiol. 2002;51(10):851–854. doi: 10.1099/0022-1317-51-10-851

28. Höcker M, Hohenberger P. Helicobacter pylori virulence factors—one part of a big picture. Lancet. 2005;362(9391):1231–1233.

18. Van der Ende A, Pan ZJ, Bart A, et al. cagA-positive Helicobacter pylori populations in China and the Netherlands are distinct. Infect Immun. 1998;66(5):1822–1826.

7. Kamakura Y, Kodama T, Gutierrez O, Kim JG, Kashima K, et al. Prevalence and correlation with clinical diseases of Helicobacter pylori cagA and vacA genotype among gastric patients from Northeast China. BioMed Research International. 2014;2014, Article ID 142980, 7 pages.

Authors’ Contributions:

FB made substantial contributions to design this study.

SA, MY, and EIA were involved in PCR and data interpretation.

AJF, SAS, and AS were responsible for sample collection and clinical databases.

FB drafted the manuscript. All authors have read and approved the final manuscript.

Authors:

1. Fehmida Bibi,
2. Sana Akhtar Alvi,
3. Sara Ali Sawan,
4. Muhammad Yasir,
5. Ali Sawan,
6. Asif A. Jiman-Fatani,
7. Sara Ali Sawan,
8. Fehmida Bibi,
9. Sana Akhtar Alvi,
10. Muhammad Yasir,
11. Ali Sawan,
12. Asif A. Jiman-Fatani,
13. Sara Ali Sawan,
14. Fehmida Bibi,
15. Sana Akhtar Alvi,
16. Muhammad Yasir,
17. Ali Sawan,
18. Asif A. Jiman-Fatani,