Original Article

The Association of cagA, vacA, babA2, babB and oipA of Helicobacter pylori with Risk of Gastric Carcinoma Development

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Abstract

Background & Objective: Helicobacter pylori (H. pylori), carried by more than half of the world population, is a major cause of chronic duodenal and gastric ulcers, gastritis and carcinoma. Colonization and toxin production include major virulence traits of H. pylori. The aim of this study was to assess the existence of H. pylori and virulence factors among patients with risk of gastrointestinal carcinoma (GC) in an Iraqi population.

Materials & Methods: During May 2016- October 2020 in Babylon, Iraq, a total of 500 biopsy samples were obtained from gastric tissue of patients with GC, gastritis, duodenitis, duodenal ulcer and gastric ulcer and cultured onto the Brucella agar. H. pylori isolates were identified using conventional biochemical and molecular tests. Molecular identification was conducted by amplification of glmM gene using the polymerase chain reaction (PCR) technique. The adhesin (babA2, babB and oipA) and toxin (cagA and vacA) genes were also amplified using PCR technique.

Results: Among 500 biopsy samples, 269 (110 from males and 159 from female patients) H. pylori isolates were identified. The age range of patients was 14-69 years (mean age=47.34±7.23). The babA2 and babB genes were detected in 59.47% and 59.10% of isolates, respectively. Notably, babA2 was observed in 89% of GC and 64% of DN strains being significantly more associated with GC and DN (<0.0001 and 0.028, respectively). Furthermore, babB-positive strains were significantly (0.042) more associated with PG. The rate of cagA and vacA was 44.60% and 48.32%, respectively. The cagA was detected in 64.73% of GC, and 100% of PG and DN strains with a significant association. We detected the oipA in 58.36% of strains which was significantly associated with GC (74%, P=0.0001), PG (88%, p<0.0001) and DN (84%, p<0.0001) as compared to oipA-negative strains.

Conclusion: The existence of H. pylori babA2, cagA and oipA virulence genes was associated with GC, DN and PG. As these genes play a crucial role in the development of gastric carcinoma, accurate control measure toward hindering the colonization of pathogenic strains is essential.

Keywords: Helicobacter pylori, colonization, virulence, gastric carcinoma, duodenal cancer

Introduction

H. pylori is colonized into the gastrointestinal tract of half of the world's population (1) in 10%-15% of cases development of acute gastritis, peptic ulcer diseases (PUD) and gastric adenocarcinoma (2, 3) H. pylori permanent colonization in the gastric mucosa (acquired from childhood) is associated with numerous gastroduodenal diseases, colorectal cancers, and MALT lymphoma with a high burden of morbidity and mortality (4). The prevalence of H. pylori in developed countries ranges 20-35%, reaching >65% in developing societies (5, 6). Bacterial virulence and genotype, host factors and epigenetics participate in the exacerbation of H. pylori infection. The bacterial colonization is mediated via some surface adhesins such as Bab, Sab,

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HopZ, AlpA and OipA protein structures. BabA mostly participates in colonization via binding to Lewis blood group receptors on the gastric epithelial cells (6, 7). Moreover, OipA is a member of Hop protein family with a high prevalence among H. pylori isolates. SabA binds to sialic acid containing glycol-conjugate domains. Presumably, SabA causes atrophy and chronic inflammation owing to substitution of Lea and Lex sialic Lewis antigens instead of non-sialic antigens. Additionally, urease enzyme causes neutralization of gastric surface and bacterial survival. H. pylori cagA gene encodes CagA protein which is mostly associated with GC and duodenal carcinoma (9). Furthermore, VacA protein causes the epithelial cells vacuolation and apoptosis (9, 10). The association of major H. pylori virulence factors with GC necessitates the screening of these genes towards recognition of populations at higher risk for GC. The objective of our study was the screening of cagA, vacA, babA2, babB and oipA genes among H. pylori isolated from patients with GC, DN and PG.

Materials & Methods

Bacterial isolates and culture conditions

Following collection of consent from 500 patients with gastritis, duodenitis, duodenal ulcer, gastric ulcer and gastro-esophageal reflux, biopsy samples were obtained during May 2016- October 2020 in Babylon, Iraq. Those patients with antibiotic therapy during previous two weeks were excluded. The samples were homogenized and cultured onto the Brucella agar (Merk, Germany) in microaerophilic conditions supplemented with 5% sheep blood, 7% calf serum (Gibco, CA, USA), 5% CO_2 and antibiotics (vancomycin, amphotericin B and trimethoprim) (Sigma Aldrich) at 37°C for 5-7 days. Phenotypic identification included catalase, oxidase, urease and nitrate tests. Molecular identification was also performed. Patients were classified as H. pylori-infected only if all the tests were positive.

Polymerase chain reaction

DNA extraction was performed using the Biospin Tissue Genomic DNA Extraction Kit (BioFlux, Japan). Molecular identification was done by amplification of glmM gene by the polymerase chain reaction (PCR). For glmM gene, PCR thermal profile was comprised of 35 cycles of denaturation (at 94 °C for 30 s), annealing (at 58°C for 30 s), extension (at 72°C for 30 s), and one final extension (at 72°C for 5 min). PCR reagents for master mix were applied as previously described (12). The adhesins (babA2, babB and oipA) and toxin (cagA and vacA) genes were also amplified using similar conditions using annealing temperatures depicted in Table 1.

Table1. Primer sequences used in this study

Primer	Sequence 5'-3'	Amplicon (bp)	Annealing (°C)	Reference
glmM	F: GGATAAGCTTTTAGGGGTGTTAGGGG R: GCTTACTTTCTAACACTAACGCGC	294	58	(13)
babB	F: ATGAAAAAAACCCTTTTAC R: CGAATTGCAAGTGATGGT	496	40	(14)
babA2	F: CCAAACGAAACAAAAAGCGT R: GCTTGTGTAAAAGCCGTCGT	271	45	(15)
oipA	F: GTTTTTGATGCATGGGATTT R: GTGCATCTCTTATGGCTTT	401	56	(16)

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cagA	F: 5′- AATACACCAACGCCTCCAAG-3′ R: 5′- TTGTTGGCGCTTGCTCTC-3′	499	60	(17)
vacA	F: 5′- GCCGATATGCAAATGAGCCGC-3′ R: 5′- CAATCGTGTGGGTTCTGGAGC-3′	259	56	(18)

Data analysis

IBM SPSS 21 was employed for data analysis considering 95% confidence interval and p value<0.05 as significant finding. Comparison of isolates carrying each virulence factor was performed with isolates lacking virulence genes using unpaired chi-square test.

Results

Demographic data

The age range of patients was 14-69 years (mean age=47.34±7.23) including 269 (110 males and 159 female) H. pylori infected patients. According to our inclusion criteria, none of patients had history of antibiotic consumption during previous two weeks. Family history of infection was observed in 179/269 (p=0.0371) of them.

H. pylori virulence genes

The rate of cagA, vacA, babA2, babB and oipA was 44.60%, 48.32%, 59.47%, 59.10% and 58.36%, respectively. The existence of all genes (in 133 isolates) was significantly associated with GC (p<0.0001), duodenal ulcer (DU, p<0.0001), pyloric gastritis (PG, p<0.0001) and duodenitis (DN, p=0.026) (Table2 and Figure1).

Table2. The existence of virulence genes in various origins

Endoscopic finding/gene	cagA	vacA	babA2	babB	oipA	p value
GC (n=83)	64.73%	55%	89%	54%	74%	0.019
DU (n=56)	63.43%	52.34%	68%	56%	69%	0.091
PG (n=121)	100%	58%	43%	61%	88%	0.041
DN (n=9)	100%	100%	64%	48%	84%	0.022

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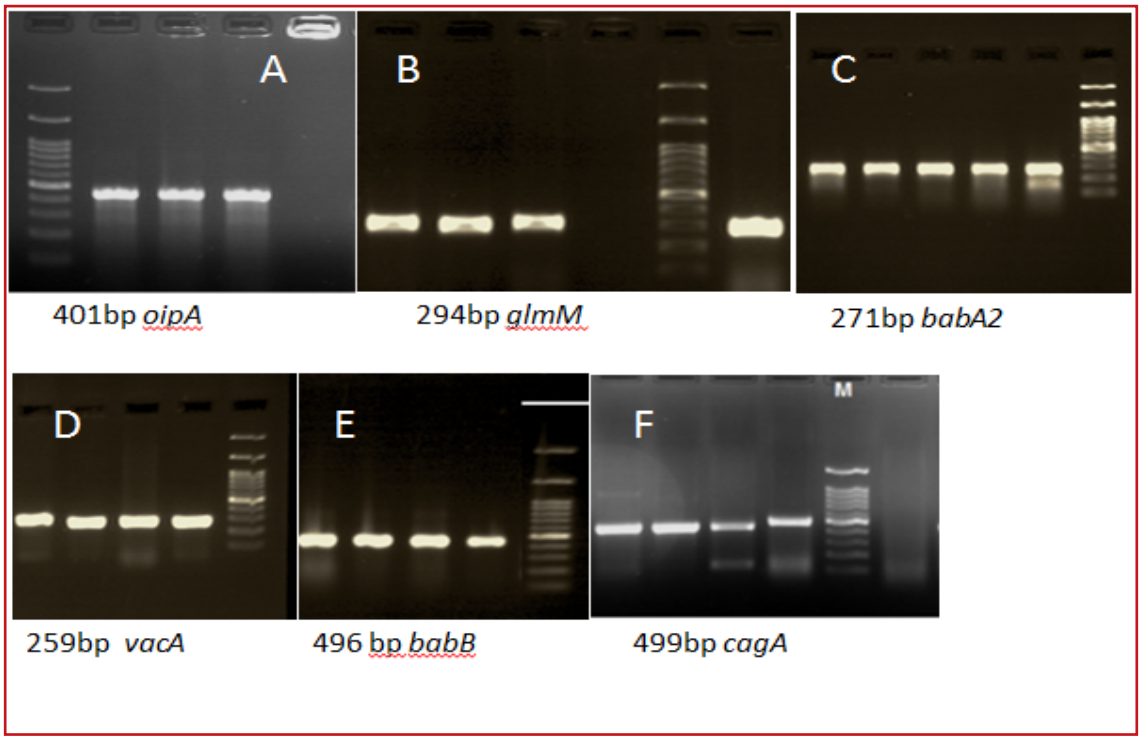


Figure1. PCR products of oipA (A), glmM (B), babA2 (C), vacA (D), babB (E) and cagA (F) on 1% gel agarose and TBE buffer using 100bp DNA marker for all genes

Discussion

H. pylori infection in human is influenced by numerous host and bacterial factors (19). Most of H. pylori strains express BabA2 (the only allelic subtype of the bab gene participating in Lewis adhesion) among those causing peptic ulcers or gastric cancer, rather than asymptomatic infection (20). The babA2 gene has a significant association in GC via induction of inflammation and persistent colonization (21). The relation of H. pylori-mediated GC and development of gastric and duodenal cancer has been proven (21). In this study, babA2 and babB were detected in 59.47% and 59.10% of isolates, respectively. Notably, babA2 was observed in 89% of GC and 64% of DN strains were significantly higher than babA2-negative strains (<0.0001 and 0.028, respectively). Furthermore, babB-positive strains were significantly (0.042) more associated with PG than negative strains. Indeed, babB gene exhibited no association with other complications. A previous study outlined that infection with babA/B mixed genotype strains amplified the risk of DU (22). The same was reported by Akbari et al. Patients with GC and peptic ulcer disease (PUD) have higher levels of the babA2 gene. The findings of this study demonstrated a link between the genotype of the babA2 gene and negative clinical outcomes such PUD and GC (23). In H. pylori, CagA is one of the most potent virulence factors. It is encoded by the cag PAI. Early in the 1990s, CagA was discovered, and is intimately linked with GC and peptic ulcers. The risk of developing gastritis and GC is higher in H. pylori strains with cag PAI compared to those without it (24). In addition, VacA is a pore-forming secreted toxin that binds to receptors such as receptor protein tyrosine phosphatase beta (RPTP). It is made up of N-terminal (p33) and C-terminal (p55) fragments (25). Helicobacter pylori secretes VacA toxins, which enhances the ability of the bacteria to colonize and contribute to gastric adenocarcinomas and peptic ulcers (26). In our study, the rate of cagA and vacA was 44.60% and 48.32%, respectively. The cagA was detected in 64.73% of GC, and 100% of PG and DN strains with a significant association. There has been a high rate of these genes

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in clinical isolates causing gastrointestinal diseases (27, 28). In another study, by analysis of virulence factors [whole genome sequencing (WGS)], Imkamp et al also revealed that 56 percent of the isolates carried the vacA s1 allele, and that 43.9 percent of the H. pylori strains were cagA-positive. It was discovered that the presence of cagA was significantly (P= 0.001) correlated with the expression of oipA, vacA s1, babA2, and hopQ allele 1. The existence of the cagA, vacA s1, and hopQ alleles was also linked to the degree of gastritis and the prevalence of H. pylori in the GC (29). However, Dabiri et al observed no association between cagA, vacA, cagE, babA2 status and clinical outcome among 160 isolates (14). Bartpho demonstrated that vacA, babA2, and oipA were predominant in gastric cancer, chronic gastritis and precancerous lesions (30). OipA encoded by the HopH gene is a protein of the outer membrane that is related to inflammation. An increased response to inflammation is produced by OipA positive Helicobacter. OipA positive H. pylori strains induce a greater degree of inflammation than OipA negative strains. Due to this, there is an increased risk of gastric cancer and peptic ulcers (31). We detected the oipA in 58.36% of strains which was significantly associated with GC (74%, P=0.0001), PG (88%, p<0.0001) and DN (84%, p<0.0001). Braga et al observed that oipA existence and functionality was associated with development of gastric diseases and in the development of gastric cancer in particular (32). In an unexpected finding, Feili et al. identified a conflicting relationship between the H. pylori oipA genotype and the risk of peptic ulcerations (PUs) and GC in Iran (32). As reported by Kianmehr et al, cagA, dupA, and babA expression are significantly higher in GC patients. Screening these genes may aid in identifying people at higher risk of gastric ulcer disease and GC (33). A major limitation of our study was lack of gene expression assessment to confirm the activity of virulence genes.

Conclusion

The existence of H. pylori virulence genes plays a crucial role in the development of gastric duodenal carcinoma which necessitates accurate control measure toward hindering the colonization of pathogenic strains.

Conflict of interest

No conflict of interest is declared by the authors.

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