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Research paper

Helicobacter pylori outer membrane protein Q allele distribution is associated with distinct pathologies in Pakistan

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ABSTRACT

Helicobacter pylori (*H. pylori*) strains expressing outer membrane protein Q (*HopQ*) promote adherence to the gastric epithelial cell. We characterized *HopQ* alleles in relation to *H. pylori*-related disease, histology and virulence markers. Gastric biopsies were obtained at esophagogastroduodenoscopy from patients with upper gastrointestinal symptoms. *H. pylori* culture, histology and polymerase chain reaction (PCR) for *HopQ* types, *cagA*, *cagA-promoter* and *vacA* alleles were performed. DNA extracted was used for PCR. Sequencing of PCR products of *HopQ* types 1 and 2 was followed by BLAST query. We examined 241 *H. pylori* isolates. *HopQ* type 1 was positive in 70 (29%) isolates, type 2 in 60 (25%) isolates, while both type 1 and type 2 in 111 (46%) *H. pylori* isolates, respectively. Nonulcer dyspepsia (NUD) was associated with *HopQ* type 2 in 48 (41%) isolates, while gastric carcinoma (GC) in 37 (53%) ($P < 0.001$) with type 1 isolates. Gastric ulcers (GU) were 39 (46%) ($P < 0.001$) in *H. pylori* infection with multiple *HopQ* alleles compared to 6 (23%) in *HopQ* type 1. Multivariate analysis demonstrated that multiple *HopQ* alleles were associated with GU OR 2.9 (1.07–7.8) ($P = 0.03$). *HopQ* type 1 was associated with *cagA* 58 (84%) ($P < 0.001$) and *cagA-promoter* 58 (83%) ($P < 0.001$) compared to 14 (23%) and 17 (28%) respectively, in type 2. *VacAs1a* was associated with *HopQ* type 1 in 59 (84%) isolates compared to *HopQ* type 2 in 35 (58%) ($P = 0.002$) isolates. *VacAm1* was associated with *HopQ* type 1 in 53 (76%) isolates compared to *HopQ* type 2 in 32 (53%) ($P = 0.004$) isolates. *H. pylori* infection with multiple *HopQ* alleles was predominant. *H. pylori* infection with single *HopQ* type 1 was associated with GC in the presence of other *H. pylori* virulence markers.

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1. Introduction

Helicobacter pylori is a Gram-negative bacterium that persistently colonizes the human gastric mucosa. Gastric colonization by *H. pylori* is a risk factor for the development of peptic ulcer disease and distal gastric adenocarcinoma (Uemura et al., 2001). When *H. pylori* isolates from unrelated humans are compared, a high level of genetic diversity is consistently detected (Hopkins et al., 1996). Genetic diversity among *H. pylori* strains helps to account for varying clinical outcomes among persons colonized with *H. pylori* (Hopkins et al., 1996). Candidate markers for distinguishing disease-associated *H. pylori* strains from less virulent strains include the presence of the cag Pathogenicity Island, BabA2 sequences, intact outer immuno proteinA (oipA) alleles, and *s1*/*m1* polymorphisms in *vacA* alleles (Achtman et al., 1999; Falush et al., 2003; Kersulyte et al., 2000; Suzuki et al., 2007; Wotherspoon et al., 1994). Moreover, the great genetic diversity in *H. pylori* is caused by high mutation rate (Kennemann et al., 2011; Morelli et al., 2010). In addition, infection with multiple strains enables recombination between strains three times more likely than by mutation (Morelli et al., 2010). Strikingly, genes encoding outer membrane proteins (OMPs) of the Hop family are at a significantly increased frequency among the imported DNA fragments (Kennemann et al., 2011). Bacterial infection of the epithelium induces a mutation burst which generates many different subpopulations of clonally related bacteria, each with a specific combination of mutated and unaffected genes. There is a selective removal of bacteria with disadvantageous mutations in essential genes from the population. Horizontal gene transfers from co-colonizing bacteria, particularly genes that encode surface components of already adapted bacteria, result in a genome that is adapted to the new host (Linz et al., 2013).

Analyses of *H. pylori* genome sequences revealed the presence of a large paralogous family of about 30 Hop genes, which encode outer membrane proteins (Ilver et al., 1998). The Hop family is the largest one and includes adhesins such as *BabA* (HopS) (Ilver et al., 1998), *SabA* (HopP) (Mahdavi et al., 2002), *OipA* (HopH) (Yamaoka et al., 2000), *AlpAB* (HopB and HopC) (Odenbreit et al., 1999) and *HopQ* (Cao and Cover, 2002). Certain *HopQ* genotypes (designated type 1 *HopQ* alleles) were found significantly more often in *cagA*-positive, type *s1-vacA* strains from patients with peptic ulcer disease than in *cagA*-negative, *s2-vacA* strains from patients without ulcer disease (Mahdavi et al., 2002). Cao et al. reported that the carriage of type 1 genotype was significantly increased in subjects with peptic ulcers in the

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US population, suggesting that *HopQ* might play a biological role in the gastric mucosa (Cao et al., 2005). Loh et al. showed that adherence to the gastric epithelial cells is facilitated in strains expressing *HopQ* in certain *H. pylori* (Loh et al., 2008). In northern Iran, *H. pylori HopQ* type 2 was found to be significantly associated with gastric cancer (Abadi and Mobarez, 2014). So far, *HopQ* allelic variation has not been analyzed in *H. pylori* strains isolated from patients in Pakistan. In the current study, we aim to characterize *HopQ* alleles in *H. pylori* strains, the relation of *HopQ* genotype to *H. pylori*-related disease e.g. gastritis, peptic ulcer, etc., histology and virulence markers e.g. *cagA* and *vacA* alleles types.

2. Material and methods

2.1. H. pylori strains

Two hundred and forty-one *H. pylori* were studied. They were cultured from 126 (52%) males and females 115 (48%), mean age of 45 ± 13 and range of 18–79 years. Patients were enrolled from the gastroenterology clinic and endoscopy unit from January 2013 to September 2014. The study was approved by the Institutional Ethics Committee and informed consent was obtained from enrolled patients. None of the patients had antibiotics, H2-receptor antagonists, proton pump inhibitors, bismuth compounds and nonsteroidal anti-inflammatory drugs in the last 8 weeks. The presenting symptoms and endoscopic findings were noted (Table 1). The diagnosis in these patients was gastritis associated with nonulcer dyspepsia (NUD) in 114(47%), gastric ulcer (GU) in 45(19%), duodenal ulcer (DU) in 39(16%) and gastric carcinoma (GC) in 43(18%). Of GC 20 (47%) were in corpus and 23 (53%) in antrum. They were all adenocarcinomas, 18 (42%) were diffuse and 25 (58%) intestinal in nature. Gastric biopsy specimens were taken from an area of inflammation in the antrum and corpus. Two biopsy specimens were used for the rapid urease test, histology and polymerase chain reaction (PCR).

Table 1
Clinical detail of the patients.*a

Age (years)	
18–45	139(58)[a]
46–79	102(42)
Gender	
Male	126(52)
Female	115(48)
Clinical feature	
Abdominal pain	186(77)
Hematemesis	45(19)
Malena	39(16)
Weight loss	43(18)
Diagnosis	
Nonulcer dyspepsia	114(47)
Gastric ulcer	45(19)
Duodenal ulcer	39(16)
Gastric cancer	43(18)
Histology	
Grade of gastritis	
Chronic active gastritis	227(94)
Chronic gastritis	14(6)
Lymphoid aggregates	
Positive	83(34)
Negative	158(66)
Intestinal metaplasia	
Positive	83(34)
Negative	158(66)
Severity of inflammation	
Mild	9(96)
Moderate	232(4)
HopQ alleles	
Type 1	70(29)
Type 2	60(25)
Types 1 and 2	111(46)

[a] Number and percentage = n (%).

Specimens for histology were dispatched in formalin and for PCR in 0.9% normal saline. The PCR for *cagA* 5′ terminal, *cagA*-promoter region, *vacA* alleles for the signal (s) i.e. *s1a*, *s1b*, *s2* and middle (m) *m1*, *m2* and *HopQ* alleles (types 1 and 2) were analyzed. *HopQ* alleles identified in *H. pylori* were either single (i.e., type 1 or type 2) or multiple types (i.e., type 1 and type 2). *HopQ* alleles were compared with demographic features, symptoms, diagnosis and histopathology as single and multiple *HopQ* alleles' types.

2.2. Bacterial culture

The specimens were transported in normal saline to isolate *H. pylori*. Each specimen was homogenized in eppendorf tubes with electric homogenizer and inoculated onto Columbia blood agar (Oxoid) medium supplemented with 10% defibrinated sheep blood and Dents supplement (containing vancomycin, trimethoprim, cefsulodin and amphotericin B) and incubated at 37 °C under microaerophilic conditions using anaerobic jars and strips producing microaerophilic conditions which are essential for the isolation and growth (Campygen strips, Oxoid, UK) for 5–7 days. Plates were then examined for bacterial growth. The identity of *H. pylori* was confirmed by typical colony morphology, Gram stain and production of urease and catalase. *H. pylori* isolates were defined as Gram-negative spiral-shaped bacilli that were catalase and urease positive.

2.3. Extraction of genomic DNA

The bacterial cells on agar plate were washed twice with phosphate buffer saline (PBS, pH 8.0) then centrifuged at 3000 rpm for 20 min. *H. pylori* DNA was extracted by a phenol/chloroform method previously described (Yakoob et al., 2000).

2.4. Histology

Gastric biopsy specimens for histopathology were stained with hematoxylin and eosin (H & E) stain for the detection of *H. pylori* and degree of gastritis as described previously (Price, 1991; Yakoob et al., 2009).

2.5. Polymerase chain reaction

2.5.1. cagA, cagA promoter, and vac A alleles

Amplification of *cagA*, *cagA* promoter, and *vacA* alleles by PCR was performed as described previously (Covacci and Rappuoli, 1996; Occhialini et al., 2001; Yakoob et al., 2011).

2.5.2. HopQ genotyping

The *HopQ* genotypes (type 1 and type 2) were determined by PCR methods (Cao and Cover, 2002). Primers and conditions used for PCR amplification of *HopQ* sequences of type 1 and type 2 *HopQ* types are shown in Table 2. To PCR-amplify *HopQ* alleles, primers used are given in (Table 2) (Cao and Cover, 2002).

2.6. Sample size

The sample size for this study was calculated to estimate the characteristics of *HopQ* alleles in Pakistani *H. pylori* strains, and its genotype in *H. pylori*-related disease e.g. gastritis, gastric and duodenal ulcer and gastric carcinoma. From previous studies *HopQ* type 1 was present in 72.5% and *HopQ* type 2 was found in 15.4% (Cao and Cover, 2002). Using a 72.5% prevalence provided a maximum sample size of 122, with 95% level of confidence and 8% bound on the error of estimation.

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