Microbial Pathogenesis 80 (2015) 67–72

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Frequency of virulence factors in *Helicobacter pylori*-infected patients with gastritis

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ARTICLE INFO

Article history:
Received 25 September 2014
Received in revised form
21 January 2015
Accepted 22 January 2015
Available online 3 February 2015

Keywords:
Helicobacter pylori
Virulence factors
Gastritis
PCR

ABSTRACT

The outcome of *Helicobacter pylori* infection has been related to specific virulence-associated bacterial genotypes. The vacuolating cytotoxin (vacA), cagA gene, oipA and babA2 gene are important virulence factor involving gastric diseases. The objective of this study was to assess the relationship between virulence factors of *H. pylori* and histopathological findings.

Material and methods: Gastroduodenoscopy was performed in 436 dyspeptic patients. Antrum biopsy was obtained for detection of *H. pylori*, virulence factors and for histopathological assessment. The polymerase chain reaction was used to detect virulence factors of *H. pylori* using specific primers.

Results: vacA genotypes in patients infected with *H. pylori* were associated with cagA, iceA1 and iceA2. In the patients with *H. pylori* infection there was a significant relationship between cagA positivity and neutrophil activity ($P = 0.004$) and chronic inflammation ($P = 0.013$) and with *H. pylori* density ($P = 0.034$). Neutrophil infiltration was found to be more severe in the s1 group than in the s2 group ($P = 0.042$). Also was a significant relationship between oipA positivity and neutrophil activity ($P = 0.004$) and with *H. pylori* density ($P = 0.018$). No significant relationships were observed between other vacA genotypes and histopathological parameters.

Conclusion: *H. pylori* strains showing cagA, vacA s1 and oipA positivity are associated with more severe gastritis in some histological features but virulence factors of *H. pylori* do not appear to determine the overall pattern of gastritis.

1. Introduction

Helicobacter pylori (*H. pylori*) is an important human pathogen that colonize the stomach. This bacterium induces gastritis, peptic ulcer and is associated with gastric carcinoma [1,2]. The clinical outcome of *H. pylori* infection has been associated with bacterial virulence factors, host gastric mucosal factors, and the environment [3]. But relationship between *H. pylori* genotype and its association with clinical outcome is not fully understood. Several possible disease-specific virulence factors have been suggested to be associated with *H. pylori* infection [4–7]. The main bacterial virulence factors include adhesins (BabA, SabA), the vacuolating cytotoxin VacA, and the products of the cag pathogenicity island (cag PAI). CagA was the most examined putative virulence factor and it is encoded by the cagA gene. A number of studies have confirmed that infection with cagA-positive strains is associated with more severe gastritis and higher prevalence of peptic ulcer and gastric cancer in western countries [8,9]. Conversely, relationship between cagA-positive status and its association with clinical outcome are not fully understood in Asian countries, where the majority of the *H. pylori* strains are cagA-positive [10–12]. The vacuolating cytotoxin A gene, which is another important virulence factor of

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http://dx.doi.org/10.1016/j.micpath.2015.01.008
0882-4010/

H. pylori, the vacA is present in all *H. pylori* strains and induces vacuolation of epithelial cells [8]. The vacA gene includes two variable parts. The *H. pylori* strains have one of two types of vacA signal sequence (s1 and s2) and two types of mid region (m1 and m2). Cytotoxin production and virulence are higher in the s1/m1 subtypes than in the s1/m2 subtype, and lower still in the s2/m2 subtype [13]. Similar cagA status, there are geographic differences between vacA status and the *H. pylori*-related diseases. In western countries infection with vacA s1 strain is more common in patients with peptic ulcer than in those with chronic gastritis. However in Asian countries, the association between vacA diversity and clinical outcome is not established [14]. BabA is an adhesion molecule that mediates the attachment of *H. pylori* to Lewis b blood group antigens on human gastric epithelial cells [15]. Three bab alleles have been identified: babA1, babA2, and babB and only the babA2 gene product is necessary for Lewis b binding activity [15]. Studies in western countries have shown that about 70% of *H. pylori* strains in Western countries were typed as babA2, which was associated with increased virulence [16]. Moreover, the triple-positive phenotype (babA2, cagA, and vacA s1) was detected at a higher frequency in isolates from patients with ulcers and adenocarcinomas, which might serve as useful markers of high-risk patients in western countries [16]. The iceA gene is induced by contact with epithelium and has two main allelic variants, iceA1 and iceA2. The presence of iceA1 allele is associated with peptic ulcer disease in western countries [17]. OipA is a proinflammatory response-inducing protein associated with high *H. pylori* density and more severe neutrophil infiltration. OipA mediates adherence of *H. pylori* to gastric epithelial cells and contributes to the pathogenesis of gastroduodenal diseases [18]. Therefore, the aim of this study was to analyze the frequency of virulence factors and to correlate the presence of babA2 with cagA, oipA and vacA, iceA genotypes of *H. pylori* strains in Iran patients and to study its association with the histologic severity of gastritis.

2. Materials and methods

2.1. Study population

The subjects included in this study were 436 patients with non-ulcer dyspepsia (NUD) (195 patients with *H. pylori* infection and 241 *H. pylori* uninfection), having recurrent abdominal pain from endoscopy unit of the Hajar Hospital in Shahrekord, Iran. From each patient, written consent was obtained and 3 biopsies were collected from gastric antrum. Two specimens were used for rapid urease test and DNA extraction, and one specimen was used for histopathology study. *H. pylori*-infection was determined by the rapid urease test, PCR (16srRNA and glmM) and histological examination of biopsies taken from the corpus. Patients were classified as *H. pylori*-infected only if the four tests were positive, respectively. This study was performed by the Ethics Committee approval No: 1025 of Shahrekord University of Medical Sciences, Shahrekord, Iran.

2.2. Histological examination

Sections of biopsy specimens were embedded in 10% buffered formalin and stained with hematoxylin and eosin to examine gastritis and with giemsa to detect *H. pylori* (Fig. 1). The histological severity of gastritis was blindly graded from normal to severe based on the degree of mononuclear cell (MNC) and polymorphonuclear leukocyte (PMN) infiltration, and atrophy according to the Updated Sydney system [19] on a four-point scale: 0, no; 1, mild; 2, moderate; and 3, severe changes.

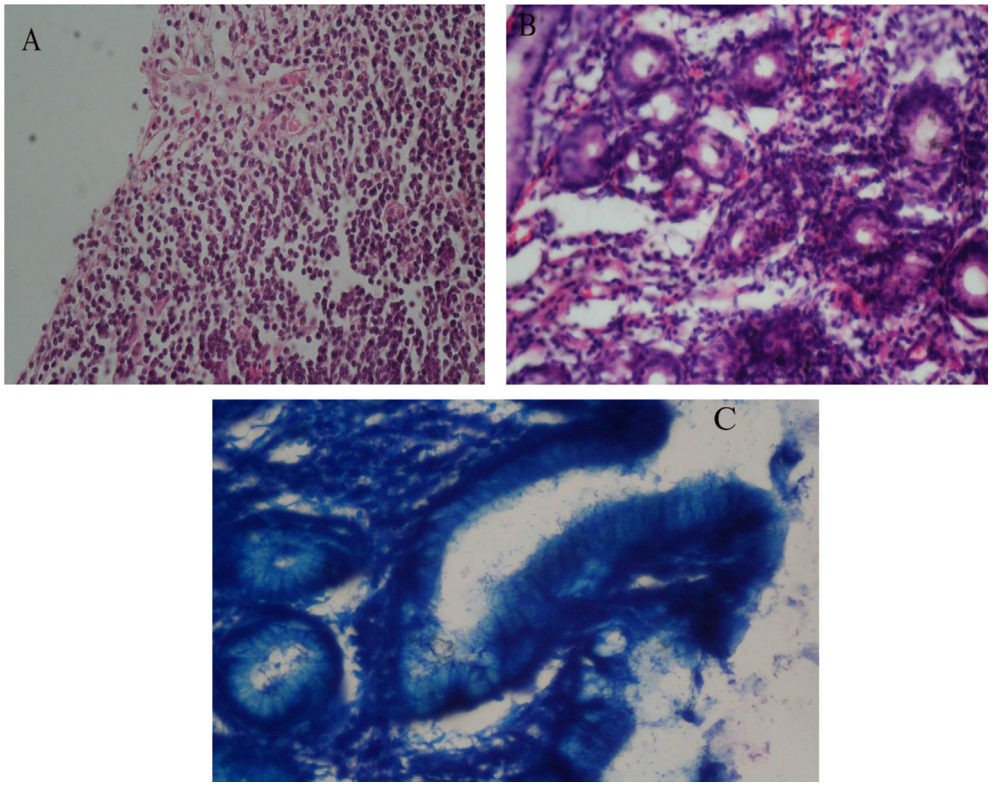

Fig. 1. Histological examination. (A) Mild chronic superficial gastritis with chronic inflammatory cells present in the superficial lamina propria in excess of normal. This is a borderline biopsy sample and illustrates the least number of cells acceptable for a diagnosis of gastritis. (B) Gastric pits infiltrated by neutrophils in a case of *Helicobacter pylori* gastritis. (C) *H. pylori* organisms present in the mucous layer on the gastric mucosal surface (400×).