Original article

Relation of bab A2 genotype of *Helicobacter pylori* infection with chronic active gastritis, duodenal ulcer and non-cardia active gastritis in Alzahra hospital Isfahan, Iran

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How to cite this article:
Safaei HG, Havaei SA, Tavakkoli H, Eshaghei M, Navabakbar F, Salehei R. Relation of bab A2 genotype of *Helicobacter pylori* infection with chronic active gastritis, duodenal ulcer and non-cardia active gastritis in Alzahra hospital Isfahan, Iran. Jundishapur J Microbiol. 2010; 3(3): 93-8.

Received: January 2010 **Accepted:** April 2010

Abstract

Introduction and objective: Bacterial virulence factors are important in determining disease outcome. The initial stage of colonization is binding of *Helicobacter pylori* to one of the gastric epithelial cells surface receptors, the Lewis b blood group antigen binding adhesion, babA. Heterogeneity among *H. pylori* strains in presence and expressing the babA gene may be a factor in the variation of clinical outcomes among *H. pylori*-infected people. We investigated the presence of babA in clinical *H. pylori* isolates and their correlation with different diseases in Iran.

Materials and methods: In the present study 81 positive culture samples out of 177 biopsies examined for the presence or absence of babA gene which were detected by PCR method. DNA extracted from 81 *Helicobacter* positive specimens, 44 chronic active gastritis, and 27 duodenal and 10 non-cardia gastric cancers.

Results: We had 58(71.6%) positive samples for babA and 23 samples were negative (28.4%) by PCR method. Relative frequency of babA genotype of *H. pylori* isolated from gastric biopsies of patients with chronic active gastritis duodenal ulcer, and non-cardia gastric cancer were 68.2%, 74.1% and 80%, respectively.

Conclusion: In our study, there was not significant correlation between the babA genotype and chronic active gastritis and duodenal ulcer (P=0.673) but significant correlation with non-cardia gastric cancer (P<0.001). Our results showed that the prevalence of babA genotype corresponds with the report from Asian countries but not with European and Latin America results.

Keywords: *Helicobacter pylori*, babA protein, *Helicobacter* infection

Introduction

Helicobacter pylori is the agent of infection associated with cancer (5.5% of all cancers) [1] and main cause of the high prevalence (40%) of all gastric cancer cases worldwide and 47% in developing countries [2-3]. Most studies reported that bacterial virulence, host genetic and environmental factors are involved in progression of diseases. Bacterial virulence factors are important in determining disease outcome. The immune system of the host attempts to clear chronic infection of *H. pylori* that brings out an excessively aggressive proinflammatory Th1 cell response resulting in a strong gastritis that precedes a series of morphological changes leading to cancer [4].

The initial stage of colonization is binding of *H. pylori* to gastric epithelial cells surface receptors, like lipids, proteins, glycolipids or glycoproteins, which lead to infiltration of inflammatory cells (neutrophils and monocytes) and possibly to the persistence of the microorganism. Once *H. pylori* reaches the epithelial layer, it adheres to the cells by using babA, SabA, AlpA, AlpB, HopZ, HpA, and other adhesions. The best-defined *H. pylori* adhesin-receptor interaction found to date is that between the Lewis blood group antigen binding adhesion, babA, which is a member of a family of *H. pylori* outer membrane proteins, and the H, Lewis b (Leb), and related ABO antigens [5]. These fucose-containing blood group antigens are found on red blood cells and in the gastrointestinal mucosa [6].

BabA is a 75- adhesion molecule that mediates the adherence of *H. pylori* to Lewis (α-1, 3/4 difucosylated) blood group antigens on human gastric epithelial cell. Three bab alleles have been identified: babA1, babA, and babB. BabA1 and babA are identical alleles except that babA1 has a 10-bp deletion of the translational initiation codon. Only the babA gene product is necessary for Lewis binding activity. Heterogeneity among *H. pylori* strains in presence and expressing the babA gene may be a factor in the variation of clinical outcomes among *H. pylori*-infected peoples [7-9].

Some studies reported babA expressing *H. pylori* are associated with more mucosal cellular inflammation and increased risk of clinical outcomes like duodenal and gastric cancer [7,10-12]. The clinical relevance of the *H. pylori* genotype has not yet been determined in clinical isolates in Isfahan, Iran. Therefore, we investigated the presence of babA in clinical *H. pylori* isolates and their correlation with clinical outcomes (chronic active gastritis, duodenal ulcer and non-cardia gastric cancer) who were referred to Alzahra hospital Isfahan, Iran.

Materials and methods

Biopsy specimens obtained from patients who underwent routine upper gastrointestinal endoscopy referring to Alzahra hospital in Isfahan, Iran. Patient's consent was obtained before the examination for additional histological and molecular analysis. One hundred seventy seven biopsies were taken from the prepyloric and incisures before receiving anti *H. pylori* treatment and according to clinical evidence by gasterologist divided to three groups, chronic active gastritis, duodenal ulcer and non-cardia active gastritis.

We took two specimens, one for rapid urease test (RUT) [2,3], and the other for culture and PCR test. In the present study from 87 positive culture samples, six cultures had contamination and excluded from investigation. DNA extracted from 81 positive culture specimens (47 male, 34 female), 44 chronic active gastritis, and 27 duodenal and 10 non-cardia gastric cancers. BabA genotype obtained from isolated

bacteria by using PCR method. The mean who were 14-88 years old was 43.

Isolation of H. pylori from biopsy samples

Biopsy specimens were cultured directly on Columbia agar medium (Merck co., Germany) supplemented with 10% fetal calf serum, 5% blood and 10µg/ml trimethoprim, 6µg/ml cefsulodin and 5µg/ml vancomycin and followed by incubation for 3-5 days at 37ºC under microaerophilic conditions. Suspected colonies identified as *H. pylori* with catalase, oxidase, urease positive tests and the appearance of Gram-negative curved bacilli. Isolates harvested for storage in Brucella broth (Merck co., Germany) contained 20% glycerol and stored at -80ºC.

Genomic DNA was extracted from the fresh isolates of bacteria before storage at -80ºC using DNA extraction kit (Roche Co., Germany) according to the manufacturer instruction. DNA density was assessed by optic densitometry. Extracted genomic DNA amplified for babA gene. PCR reactions were performed in a final volume of 50µL containing 5µL 10x buffer + Mg^{2+}, 2mM/L dNTP, two unit Taq DNA polymerase, 100ng from genomic DNA as a template, and 25 Pico mole from each primers. The primers were b*abA*F (5'-AATCCAAAAAGGAGAAAAAGTATGAAA-3') and b*abA*R (5'-TGTTAGTGATTTCGGTGTAGGACA-3') for babA amplification and product size was 833 bp(16). PCR was performed using a thermal cycler (Eppendorf, Europe) under the following conditions: an initial denaturation for 10min at 94°C; 35 cycles of 1min at 94°C, 1min at 55°C, and 1min at 72°C; and a final extension at 72°C for 10min [8]. PCR yields electrophoresed in 1.5% agarose gel (Roche, Germany) containing Ethidium bromide. DNA ladder (Roche Co., Germany) was used to detect the molecular weights of observed bands under UV lamp [2].

Statistics

Results were analyzed using Pearson chi-square for assessing of relationship between babA genotype and sex and age with duodenal ulcer, chronic active gastritis and non- cardia gastric cancer.

Results

DNA extracted from 81 (47 male, 34 female) *H. pylori* positive culture specimens out of 177 biopsies were taken, including 44 chronic active gastritis, 27 duodenal ulcers and 10 non-cardia gastric cancers (Fig. 1). Average age in chronic active gastritis, duodenal ulcer and non-cardia gastric cancer with *Helicobacter* infection were 41, 47, and 65 years old respectively, (Fig. 2). babA genotype was obtained from isolated bacteria by using PCR method. We had 58 (71.6%) positive samples for babA and 23 were negative ones (28.4%). Relative frequency of babA genotype of *H. pylori* isolated from gastric biopsies of patients with chronic active gastritis duodenal ulcer, and non-cardia gastric cancer were 68.2%, 74.1% and 80%, respectively.

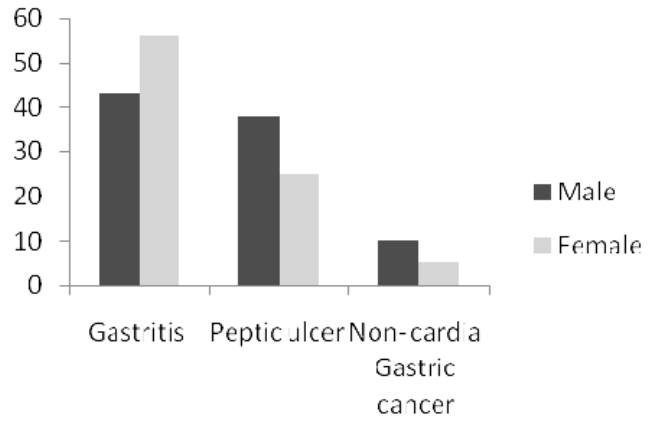

Fig. 1: Frequency of *H. pylori* infection in chronic active gastritis, duodenal and non-cardia gastric cancer according to sex

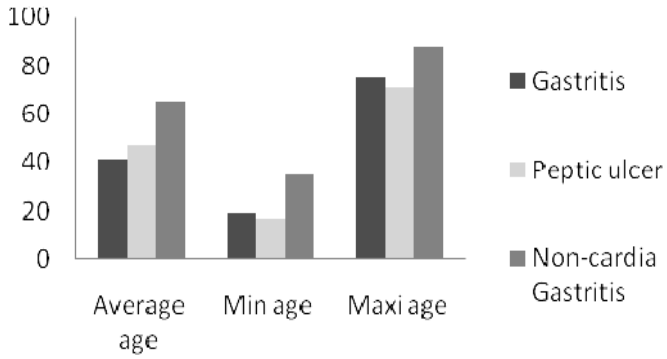

Fig. 2: Distribution of *H. pylori* infection according to age in chronic active gastritis, duodunal culcer and non-cardia gastric cancer

Discussion

There is continuing interest in identifying *H. pylori* virulence factors that might predict the risk for symptomatic clinical outcomes. It has been proposed that Lewis b blood group antigen binding adhesion is one of those markers. Its association with clinical disease remains controversial. The capability of many *H. pylori* strains to attach fucose-containing blood group antigens of gastric mucosa by specific adhesion, babA, might contribute to virulence, development of diseases and the persistency of *H. pylori* infection. The putative virulence factor, the outer-membrane protein babA (blood group antigen binding adhesion) has been reported to be associated with peptic ulcers and gastric cancer [6-8].

Fucosylated ABO blood group antigens were identified by babA adhesion protein of *H. pylori*. Two corresponding genes encoding babA have been cloned and named babA1 and babA2. Only the babA2 gene is functionally active [9]. Many studies showed that *Helicobacter* expressing babA are associated with more severe mucosal cellular inflammation and increased risk of clinical outcome diseases like active gastritis, duodenal ulcer and non-cardia gastric cancer, however it is controversial and some studies disagree [6,12-13].

The study of Grehard *et al.* [8] indicated the presence of babA was significantly associated with duodenal ulcer (P=0.0002) and adenocarcinoma (P=0.033) and would be a useful marker to identify patients who are at higher risk for specific *H. pylori*-related diseases. The distribution of the presence of the babA genotype in Western countries has been shown to be about 66 to 72% in different studies [9,14]. The present study showed that the prevalence of the babA genotype is 71.6% and in gastritis 68.2 %, duodenal ulcer 74.1% and in non-cardia gastric cancer 80%.

In our study there is not a significant correlation between the babA genotype and clinical outcome (P=0.673) but there was a significant correlation between non-cardia gastric cancer and presence of babA genotype (P<0.001). Kashani *et al*. [15] reported that babA prevalence in gastric cancer (GC), non-ulcer dyspepsia (NUD) and peptic ulcer disease (PUD) patients were 75%, 47.7% and 33.3% respectively in Tehran, the capital city of Iran. The results of this study are not in accordance with those in Italy and Brazil [10,16]. However, regarding chronic active gastritis and duodenal ulcer, The results obtained in China is similar to ours but their results are different from ours in non-cardia gastric cancer [17]. European countries such as Finland, Germany, Portugal results showed a correlation between babA genotype and duodenal ulcer but this was not the case Sweden [18-19]. Molecular epidemiological data have gradually revealed more details of the geographical distribution of *H. pylori* genetic variations [14,20].

The study in Japan suggested that the prevalence of the babA genotype is higher in Japan than in Western countries and that there is not a significant correlation between the babA genotype and clinical outcome in Japan. These results are not in accordance with those of a recent study in a Western population [21]. It is difficult to explain the different clinical outcomes from virulence factors only by babA2 of *H. pylori*. It should be noted that other virulence factors of *H. pylori* might also account for some of the differences in clinical out comes incidence.

The variation between these reports and the results in the present study may be due to patients' selection and the geographic origin of the patients, which may play an important role. In addition, we described association between presence of the babA gene and age and sex of patients. With regards to the relationship between babA gene and sex of patients, there was no significant relationship (P>0.05, Fig. 1). Mean age of patients with chronic active gastritis, duodenal ulcer and non-cardia gastric carcinoma were 41, 47 and 65, respectively. Only in cancer group in comparison to two other groups, there was significant differences (P<0.001, Fig 2). Data analysis using logistic regression showed that there was no statistically significant relationship between presence of the babA gene and sex, age and diseases of patients and none of these factors could predict whether the *H. pylori* genotype is negative or positive.

Conclusion

In conclusion, our data do not support the hypothesis that the virulence factors of *H. pylori*, babA are strongly associated with gastritis and peptic ulcer diseases but there is a relation with non-cardia gastric cancer in our country. Further study is necessary to predict the other genes effect clinical outcomes in our local area.

Acknowledgement

This study was financially supported by the vice chancellor for research affairs of the Isfahan University of Medical Sciences, Isfahan, Iran (Grant No. 83173).

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