

Association Between *H. pylori* *babA* Virulence Factor with Clinical Outcome and ABO Blood Groups

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Helicobacter pylori infection is a prevalence infection 50% of the human population. The main *H. pylori* adhesin is the BabA, which was the first identified factor. The aim of this study was to determine the relationship between the ABO blood groups and various gastrointestinal diseases 140 patients, were included in this study. Gastric biopsies were taken for recognition of *H. pylori* by RUT and PCR. Blood samples were tested for ABO blood group. In the present study 140 *H. pylori* positive samples examined for the presence or absence of *babA* gene by PCR. From 140 patients, 35% were positive for *babA* gene and 65% were negative for this gene. Positivity rate of *H. pylori babA* infection was 42.4 % in blood group O, 18.8 % in blood group A, 100% in blood group B and 44.8 % in blood group AB. The frequencies of ABO blood group among endoscopic findings are predominant for Gastritis for group A. In our study, There was statistically significant difference in *babA* (+) and *babA* (-) were compared in endoscopy finding ($P < 0.001$) and there was statistically significant difference in positivity rate of *H. pylori* infection among ABO blood groups ($p < 0.001$). The higher incidence of Gastritis and peptic ulcer was in patients with blood group A and AB and there was statistically significant between these symptoms ($p = 0.02$). Our results showed that the prevalence of *babA* genotype is associated with gastritis and gastric ulcer and there is a relation with ABO blood group.

Key words: *Helicobacter pylori*, *babA*, ABO blood groups, gastrointestinal, Iran.

Helicobacter pylori (*H. pylori*) is a gram-negative flagellated bacteria that colonizes in human stomach^{1,2}. *H. pylori* infection is a high prevalence infection more than 50% of the human population and it is frequent in developing countries³. *H. pylori* is a most important risk factor for persistent gastritis, peptic ulcer and gastric

cancer⁴. The blood groups antigens present on Red Blood Cell (RBC) are biological markers of any individual that distribution these markers vary in populations the worldwide^{5,6}. Many studies indicated an association between blood groups and *H. pylori* infection⁷, so many studies have not shown such an association⁸. The ABO phenotype has been related with stomach ulcers, which are more common in individuals with O phenotype and in A individuals gastric cancer is seen frequent⁹.

The main *H. pylori* adhesin is the blood group antigen-binding adhesin A (BabA), which

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was the first identified adhesion factor in *H. pylori*^{10, 11}. BabA causing the binding of the bacterium to the fucosylated Lewis b blood group antigen, Le^b^{12, 13} and related terminal fucose found on blood group O (H antigen), A and B antigens¹⁴ which are expressed on the surface of mucins and gastric epithelial cells¹⁵. Only the *babA* gene product is essential for Lewis binding activity. Heterogeneity between *H. pylori* strains in existence and also expressing the *babA* gene may be a factor in the difference of clinical outcomes among *H. pylori*-infected individuals^{16, 17}. Some studies reported *babA* expressing *H. pylori* are related with more mucosal cellular inflammation and increased possibility of duodenal and gastric cancer^{18, 19}.

Therefore, the aim of this study was to determine the relationship between the ABO blood groups and various gastrointestinal diseases in infected patients by *H.pylori* *babA* positive virulence factor in west of Iran.

MATERIALS AND METHODS

Sampling

In this study, 140 patients with peptic ulcer (PU), gastric ulcer (GU) or duodenal ulcer (DU) included and informational questionnaires filled out by each patient and clinical assessments were performed and the gastrointestinal signs and symptoms were recorded. Gastrointestinal endoscopy was done according to standard medical method. In addition written consent obtained before biopsy sampling from the antrum. The patients were 90 males and 50 females whose age ranged from 18-75 years. Symptoms gastritis is acute and chronic inflammatory cells infiltration with degeneration and detection of microorganisms. One section of the specimens was used for the rapid urease test (RUT) immediately after collection. The remained biopsy specimens were placed in tube for pathological exam and polymerase chain reaction (PCR).

Rapid Urease Test

Rapid urease test carried out for diagnosis of *Helicobacter pylori* infection. The urease created by *H. pylori* hydrolyzes urea to ammonia, which increases the pH of the solution, and changes its color from yellow (negative) to red (positive).

DNA extraction & PCR amplification

Genomic DNA was extracted directly from all biopsy samples by using DNA extraction kit (Bioflux, Japan) according to the manufacturer instruction. For *H.pylori* positive samples detection, PCR was performed on genomic DNA from gastric biopsies directly using *H. pylori* 16SrRNA specific primers in previous study^{20, 21}. The identification of the isolates has been confirmed by using the primer of the 229 pb. PCR amplification conditions were carried out in 25 µl of reaction mixture containing 0.5 µl of each primers(10PM), 0.5 µl dNTP mix(10mM), 1 µl MgCl₂(50mM), 0.1 µl Taq polymerase(5unit/µl), 2 µl genomic DNA. The total volume was adjusted to 25 µl by ddH₂O. Thermal cycling conditions for amplifying markers were as follows: 95°C for primary denaturation for 5 min, 35 cycles of 94 denaturation for 30 seconds, 54°C annealing temperature for 30 seconds, 72°C extension for 30 seconds and 72°C final extension for 5mins. After amplifying desired piece of DNA, PCR products separately were run in 8% polyacrylamide gel electrophoresis at 35-40 mA for 3h, following silver staining of the gel, DNA bands were visualized.

H.pylori *babA* genotyping

The identification of the *H. pylori* *babA* gene, was achieved by specific primer according to the previously published study^{20, 21}. Thermal cycling conditions for containing: 95°C for primary denaturation for 5 min, 8 touchdown cycles of 94°C denaturation of DNA strands for 30 seconds, 55°C to 62°C annealing temperature for 30 seconds, 72°C extension for 30 seconds and in next 27 cycles of 94 denaturation for 30 seconds, 60°C annealing temperature for 30 seconds, 72°C extension for 30 seconds and 72°C final extension for 5mins. In each PCR, 0.3 µl of each primers (10PM), 0.5 µl dNTP mix(10mM), 2 µl MgCl₂(50mM), 0.1 µl Taq polymerase (5unit/µl), 2 µl genomic DNA and total volume was adjusted to 25 µl by ddH₂O. Specific amplified fragments containing 286 bp related to the *babA* gene were separated by electrophoresis on a 8% polyacrylamide gel and visualized using silver staining.

Determination of ABO blood group

1 cc peripheral blood in tubes containing 0.5 M EDTA from all patients was obtained for determination of ABO blood group antigens by a standard hemagglutination test. The ABO blood

group determination carried out by the conventional hemagglutination test using the anti-A and anti-B sera. The ABO blood phenotyping procedure is based on the principle of agglutination or aggregation as the patient's blood is reacted with anti-A, anti-B antibodies separately.

Statistical analysis

Statistical analysis was carried out by Statistical Package for Social Science (SPSS) version 20. The relationship between the factors was determined using Chi-Square (X²) test. P value less than 0.05 was considered as statistically significant and P- value over than 0.05 considered as non-significant.

RESULT

From 140 patients *H. pylori* positive were enrolled in study, 90 (64%) were males and 50 (36%) were females. The age range of patients was 18-75 years with mean 40 years old. The distribution of the ABO blood groups of the patients was A (49.28%) followed by O (23.57%), AB (21%) and B (6.42%). The rate of *H. pylori* infection increase with age. Of these patients 49 (35%) were positive for *babA* gene and 91 (65%) were negative for this gene. Based on endoscopy results, there were more patients suffering from Gastritis (n = 91; 65%) than those with GU (n = 25; 17.8%) and DU (n = 10;

Table 1. Relationship between clinical outcome and *babA*

Clinical outcome	Patients number	<i>babA</i> positive (%)	<i>babA</i> negative (%)
Gastritis	91	19 (20.8)	72 (79.12)
GU	25	13 (52)	12 (48)
DU	14	7 (50)	7 (50)
Gastritis & ulcer	10	10 (100)	-

Table 2. Rate of ABO blood group among *H.pylori babA* positive and negative patients

Clinical outcome	Patients number	<i>babA</i> positive (%)	<i>babA</i> negative (%)
A	69	13 (18.9)	56 (81.1)
B	9	9 (100)	-
AB	29	13 (44.9)	16 (55.1)
O	33	14 (42.5)	19 (57.5)

Table 3. Association among endoscopy sign and blood groups.

ABO blood group	Gastritis (%)	GU (%)	DU (%)	Gastritis & ulcer (%)
A	53 (76.8)	9 (13)	5 (7.2)	2 (2.9)
B	6 (66.7)	3 (33.3)	-	-
AB	11(37.9)	7 (24.13)	6 (20.7)	5 (17.24)
O	21 (63%)	6 (18.2)	3 (9.0)	3(9.0)

10%) and There was statistically highly significant difference observed when *babA* (+) and *babA* (-) patients were compared in endoscopy finding (P<0.001)(Table1).

In this study, the A blood group (n = 69; 49.3%) was prevalent over other ABO blood groups (O: n = 33, 23.5%; B: n = 9, 6.4%; AB: n = 29, 21.0%). The distribution of ABO blood groups and *H. pylori babA* infection demonstrated that positivity

rate of *H. pylori babA* infection was 42.4% in blood group O, 18.8% in blood group A, 100% in blood group B and 44.8% in blood group AB (Table 2). Thus the frequency of blood group O and AB in *H. pylori babA* infected patients was the highest and blood group A was the lowest. There was statistically highly significant difference in positivity rate of *H.pylori* infection among ABO blood groups (p< 0.001). The pattern in case of

negatively *babA* patients with A being the highest and B the lowest (none) (Table 2).

Table 3 shows that the distribution of endoscopic findings in patient and relation with ABO blood group. The frequencies of ABO blood group among endoscopic findings (Gastritis vs DU and GU): 53 (76.8%) versus 14 (20.2%) for group A, 6 (66.6%) versus 3 (33.3%) for group B, 11 (37.9%) versus 13 (40.9%) for group AB, and 21 (63%) versus 9 (27%) for group O. The higher incidence of Gastritis was in patients with blood group A and higher rate of peptic ulcer was in patients with AB blood group compared to others, although there was statistically significant between these symptoms ($p = 0.02$) in respect to other blood group phenotypes.

DISCUSSION

In this study we investigated relationship between *babA* genotype and clinical outcome and ABO blood group also association ABO blood group and clinical outcome. In our study there was a significant association between the *babA* genotype and clinical outcome ($p < 0.001$) and there was a significant association between ABO blood group and presence of *babA* genotype ($P < 0.001$). The virulence factor, the outer membrane protein *babA* (blood group antigen binding adhesion) has been reported to be associated with peptic ulcers and gastric cancer⁶⁻⁸, this virulence factor (*babA*) causes binding of bacteria to gastric epithelium to and allow persistent colonization²². Many studies showed that *H. pylori* expressing *babA* are associated with more severe mucosal cellular inflammation and increased risk of active gastritis, duodenal ulcer and noncardia gastric cancer, however it is debatable and several studies have different results^{11, 23, 24}. The study of Grehard *et al.* indicated the presence of *babA* was notably associated with duodenal ulcer and adenocarcinoma and would be a valuable marker to recognize patients who are at higher risk for specific *H.pylori*-related diseases¹⁷. The current study showed that the frequency of the *babA* genotype is 35% and in gastritis 21%, gastric ulcer 50%, duodenal ulcer 52% and in Gastritis & ulcer 100%. In our study there is a significant correlation between the *babA* genotype and clinical outcome. In Iran Kashani *et al.*,²⁵ showed that *babA* incidence

in gastric cancer (GC), non-ulcer dyspepsia (NUD) and peptic ulcer disease (PUD) patients were 75%, 47.7% and 33.3% respectively. The results of this study are not in agreement with those in Italy and Brazil^{26, 27}. But, regarding chronic active gastritis and duodenal ulcer, the outcome obtained in China is comparable to our study²⁸. European countries such as Finland, Germany, and Portugal results showed a association between *babA* genotype and duodenal ulcer but this was not the case Sweden^{29, 30}.

The ABO blood group antigens confer resistance against definite infectious disease³¹. The risk of gastric cancer was found higher among those with the blood group A while the gastric ulcer was frequent in blood group O individuals^{32, 33}. Studying the distribution of *H. pylori* *babA* genotype and their clinical finding between patients reveal that *babA* genotype positivity among patients with gastritis, gastric ulcer, duodenal ulcer, and Gastritis & ulcer was 39%, 26.5%, 14% and 20%, respectively. These results showed the difference was statistically significant ($P < 0.001$) between *babA* genotype positivity and degree of clinical status of these patients. The results of this study showed that there was a significant association between ABO blood groups and *babA* genotype positivity, in which type B has a greater tendency towards infection and type A to non-infection. These results are different with data obtained from other researchers showing the greater susceptibility of blood group O to *H.pylori* infection^{7, 34}. While the this results agree with some previous studies which demonstrated that the O blood group did not represent a risk factor for *H. pylori* infection^{35, 36}. Previous studies verified that blood group O is associated with duodenal ulcer disease, although gastric ulcer and gastric carcinoma are associated with blood group A^{37, 38}. In Romshoo *et al.* study, the majority patients with peptic ulcer (56%) had blood group O and it though a risk factor for peptic ulcer³⁹. In another study of Swedish and Danish blood donors have demonstrated that individuals with blood group O have a higher risk of peptic ulcers than those with non O blood groups⁴⁰. Those findings have been confirmed by many other studies^{41, 42}. The present study show higher prevalence of Gastritis in patients with blood group A compared to other clinical symptom (76.8% vs 23%) , although is

statistical difference between diseases in blood group A in respect to other blood groups , $p=0.02$. The difference between these reports and the results in the this study may be due to type selection of patients and the geographic region of the sampling, which may play an key role and also This is probably due to number of patients enrolled in this study.

CONCLUSION

In conclusion, our data support the assumption that the virulence factors of *H. pylori*, *babA* are associated with gastritis and gastric ulcer diseases but there is not a relation with *H. pylori*, *babA* genotype and ABO blood group. Additional study with more patients necessary to predict exact effect clinical outcomes and correlation with type of blood group in our area and other region.

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