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Associations between seroprevalence of *Helicobacter pylori* and ABO/rhesus blood group antigens in healthy blood donors in southwest Iran

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Abstract

Objective: To investigate correlations between ABO/rhesus (Rh) blood group antigens and anti-*Helicobacter pylori* and anti-cytotoxin-associated gene A (CagA) seropositivity in blood donors.
Methods: A total of 311 blood donors were enrolled. ABO and Rh blood groups were determined using hemagglutination tests. Specific anti-*H. pylori* IgG and anti-CagA IgG antibodies in sera were quantitated by enzyme-linked immunosorbent assay. Correlations between blood groups and anti-*H. pylori* and anti-CagA seropositivity were evaluated using the Chi-square test.

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Results: O+ was the most frequent blood type (38%, n = 118). Anti-*H. pylori* IgG seropositivity was observed in 240 (77.2%) blood donors, while anti-CagA IgG seropositivity was observed in 132 (42.5%) blood donors. Although seropositivity rates for both anti-*H. pylori* and anti-CagA IgG were higher in individuals with blood type O, no statistically significant associations were observed between seropositivity and any ABO/Rh blood groups.
Conclusion: Individuals with blood type O may have higher rates of *H. pylori* seropositivity.

Keywords
Helicobacter pylori, cytotoxin-associated gene A, ABO blood group, enzyme-linked immunosorbent assay, Iran, seroprevalence

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Introduction

Helicobacter pylori infection affects more than half of the human population worldwide.[1] The infection may occur during childhood and persist throughout life. Infection remains asymptomatic in most individuals, although some individuals develop acute gastritis, peptic or duodenal ulcers, gastric cancer, and mucosa-associated tissue lymphoma.[1] Gastric cancer is the fifth most prevalent cancer worldwide and the third leading cause of cancer deaths.[2] The transmission mechanisms of *H. pylori* remain unclear; however, the reservoir of the bacterium is the human stomach. The organism is transmitted vertically or horizontally from human to human including within families and through exposure to environmental contamination. Recent studies have shown that transmission of the organism between sexual partners can occur.[3,4]

H. pylori possesses several virulence factors including urease (*UreA*), outer membrane proteins, cytotoxin associated gene A (*cagA*), vacuolating cytotoxin gene A (*vacA*), induced by contact with epithelium (*IceA*), blood group antigen binding adhesin (*BabA*), and outer inflammatory protein (*OipA*).[5] Among these virulence factors, *cagA* and *vacA* genes are the most frequently identified in clinical isolates. CagA is a powerful bacterial toxin that is associated with gastritis and gastric cancer.[5] The *cagA* gene is located on the *cag* pathogenesis island, which produces a type 4 secretory system. The CagA protein is injected into host cells via this system, leading to induction of cytokine production. The *cagA* gene can cause considerable inflammation and severe infection in the gastrointestinal system and has been associated with conditions such as peptic ulcer and gastric cancer.[6,7]

Several diagnostic methods are used to identify *H. pylori* infections including: (i) invasive methods (e.g., endoscopy and culture) followed by histopathology and/or various molecular and nucleic acid amplification tests; and (ii) non-invasive endoscopy-independent methods such as urea breath tests, fecal antigen tests, and serological tests.[8] Among non-invasive methods, serological tests are simple, rapid, inexpensive, and convenient. Many studies recommended using serological tests such as screening of serum immunoglobulin G (IgG) antibodies by enzyme-

linked immunosorbent assay (ELISA) for epidemiological research purposes.[9,10]

The ABO blood group system remains the most important factor in human blood transfusion. This system is controlled by the ABO gene that modifies the carbohydrate content of red blood cell antigens. The ABO gene located on chromosome 9q34 encodes a glycosyltransferase that catalyzes the transfer of nucleotide sugars to the H antigen and organizes the production of ABO blood group antigens.[11]

Several studies have proposed that ABO/rhesus (Rh) blood types may affect the development of infectious and non-infectious diseases including hepatitis B, *H. pylori* infections, cancers, and gastroduodenal ulcers.[11–13] In a previous study, a high prevalence of O blood group was observed among patients with gastroduodenal ulcers.[11]

Although associations between ABO blood groups and risk of *H. pylori* infection have been investigated in several studies, a meta-analysis by Chakrani et al.[13] showed conflicting results in this regard. Further studies are required to understand the links between blood groups and *H. pylori* infection. Thus, this study aimed to evaluate associations between ABO/Rh blood groups and anti-*H. pylori* and anti-CagA seropositivity in blood donors referred to blood transfusion centers in Ahvaz city, southwest Iran.

Materials and methods

Ethics approval

This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (IR.AJUMS.REC.1396.370). The study protocol followed the principles laid out in the Declaration of Helsinki. Written informed consent was obtained from all participants.

Study population

This cross-sectional study investigated associations between ABO/Rh blood groups and *H. pylori* seropositivity in healthy blood donors referred to transfusion centers in Ahvaz, Iran, between July 2018 and April 2019. The participants were interviewed by a physician using a questionnaire that included questions regarding sociodemographic characteristics (age, sex, and smoking history) and clinical characteristics (history of *H. pylori* infection, history of treatment for *H. pylori* infection, and any comorbidities). According to the regulations for blood donation in Iran, all blood donors are thoroughly examined clinically and using a systematic questionnaire to assess the presence of any individual or family diseases. Donors with no comorbidities and no history of allergies, cardiovascular disease, seizures or epilepsy, drug addiction, surgery, tattooing and cupping, and jaundice were considered healthy. The inclusion criteria were: weight more than 50 kg; no history of hypertension, heart disease, or jaundice; no history of high-risk sexual behaviors; no cupping and tattooing; and no drug addiction. Participants who did not meet these eligibility criteria were excluded.

ABO blood grouping

Whole blood (5 mL) was obtained from each donor using an ethylenediaminetetraacetic acid-containing vacuum tube for ABO blood grouping. Another 5-mL sample of whole blood was obtained in a clot activator tube for serum preparation. To separate serum, the clot activator tubes were centrifuged at $1500 \times g$ for 10 minutes. ABO and Rh blood group determination was performed by cell grouping forward typing using anti-A, anti-B, and anti-D antibodies (Iranian Blood Research and Fractionation Co., Tehran, Iran). For

ABO blood group reverse typing, serum grouping was also performed using anti-A and anti-B antibodies.

*Detection of anti-*H. pylori *antibodies*

All serum samples were analyzed for the presence of anti-*H. pylori* IgG and anti-CagA IgG antibodies using commercial *H. pylori* specific ELISA kits (EUROIMMUN Medizinische Labordiagnostika, Lübeck, Germany) according to the manufacturers' instructions. The cut-off values for both tests were 20 relative units/mL.

Statistical analysis

Data were analyzed using SPSS version 20 (SPSS Inc., Armonk, NY: IBM Corp). The chi-square test was used to assess differences between groups. Values of $P < 0.05$ were considered statistically significant.

Results

Among 395 blood donors, 311 individuals met the inclusion criteria for participation in this study. Tables 1 and 2 show demographic data for all blood donors and the prevalence of anti-*H. pylori* IgG and anti-CagA IgG seropositivity in each ABO blood group, respectively. The participants included 304 (97.8%) men and 7 (2.2%) women with a mean age of 40.8 years (range: 20 to 62 years). The results of ABO and Rh blood grouping showed that O+ was the most frequent blood group (n = 118, 38%) followed by B+ (n = 76, 24.4%), A+ (n = 71, 22.8%), AB+ (n = 28, 9%), A− (n = 7, 2.3%); AB− (n = 4, 1.3%), B− (n = 4, 1.3%), and O− (n = 3, 1%).

A total of 249 (80.1%) blood donors tested positive for either anti-*H. pylori* IgG or anti-CagA IgG, while 62 (19.9%)

Table 1. Demographic characteristics of blood donors participating in this study.

Characteristic	Anti-*Helicobacter pylori* IgG seropositive (n = 240)	Anti-CagA IgG seropositive (n = 132)	All participants (n = 311)
Sex (M/F), n	240/0	131/1	304/7
Age (years), mean ±SD	40.9 ± 8.8	40.1 ± 8.6	40.8 ± 8.8
Cigarette smoking, n (%)	42 (17.5%)	21 (15.9%)	52 (16.7%)
History of *Helicobacter pylori* infection, n (%)	9 (3.8%)	7 (5.3%)	23 (7.4%)

Abbreviations: M, Male; F, female; IgG, immunoglobulin G; CagA, cytotoxin-associated gene A; SD, standard deviation.

Table 2. Prevalence of anti-*Helicobacter pylori* IgG and anti-CagA IgG seropositivity by ABO/Rh blood group.

Blood group	Anti-*Helicobacter pylori* IgG seropositivity, n (%)	Anti-CagA IgG seropositivity, n (%)	Anti-*Helicobacter pylori* IgG/Anti-CagA IgG seropositivity, n (%)	All participants, n (%)
A	61 (25.4%)	35 (26.5%)	34 (27.6%)	78 (25.1%)
B	64 (26.7%)	31 (23.5%)	28 (22.8%)	80 (25.7%)
AB	24 (10.0%)	15 (11.4%)	13 (10.6%)	32 (10.3%)
O	91 (37.9%)	51 (38.6%)	48 (39.0%)	121 (38.9%)
Rh+	222 (92.5%)	122 (92.4%)	113 (91.9%)	293 (94.2%)
Rh−	18 (7.5%)	10 (7.6%)	10 (8.1%)	18 (5.8%)
Total	240 (100%)	132 (100%)	123 (100%)	311 (100%)

Abbreviations: IgG, immunoglobulin G; CagA, cytotoxin-associated gene A; Rh, rhesus.

tested negative for both markers. A total of 123 (39.5%) participants tested positive for both markers. Nine (2.9%) donors were positive for only anti-CagA IgG, while 117 (37.6%) donors were positive for only anti-*H. pylori* IgG. Anti-*H. pylori* IgG seropositivity was observed in 240 (77.2%) participants. We observed no significant associations between mean age and anti-*H. pylori* IgG seropositivity. Fifty-two (16.7%) donors smoked cigarettes while 259 (83.3%) did not smoke cigarettes. The Chi square test revealed statistically significant differences in anti-*H. pylori* IgG seropositivity in smokers ($P = 0.008$) compared with non-smokers.

A history of *H. pylori* infection was identified in 23 (7.4%) donors. Among these participants, 21 (6.7%) had been treated for gastric ulcers, while 2 (0.7%) had not received treatment for *H. pylori* infection. The remaining 288 (92.6%) participants had no history of *H. pylori* infection (Table 1).

Although the prevalence of anti-*H. pylori* IgG seropositivity was higher in individuals with blood type O (37.9%, n = 91/240), no statistically significant associations were observed between seropositivity and any ABO blood groups. The prevalence of anti-*H. pylori* IgG seropositivity in donors of other blood groups was as follows: B (26.7%, n = 64), A (25.4%, n = 61), and AB (10.0%, n = 24) (Table 2). There was no statistically significant difference in the prevalence of anti-*H. pylori* IgG among Rh-positive and Rh-negative donors.

Anti-CagA IgG was positive in 132 (42.5%) blood donors. The prevalence of anti-CagA IgG seropositivity by ABO blood group was as follows: O (38.6%, n = 51), A (26.5%, n = 35), B (23.5%, n = 31), and AB (11.4%, n = 15) (Table 2). Thus, the highest prevalence of both anti-*H. pylori* IgG and anti-CagA IgG antibodies was observed in donors of blood type O and the lowest prevalence was observed in donors of blood type AB. No significant association was observed between the prevalence of anti-CagA IgG seropositivity and any blood group. There was no statistically significant difference in prevalence of anti-CagA IgG among Rh-positive and Rh-negative donors. The prevalence of anti-CagA IgG antibodies in smoking donors was higher than that in non-smokers ($P = 0.019$).

Discussion

Recently, data from experimental studies and epidemiological surveys have suggested a potential role of ABO blood groups as risk factors for *H. pylori* infection.[13] Some studies have reported positive associations between *H. pylori* infection in individuals with blood type O, while other studies have shown no significant associations.[13] Only a few studies have evaluated the seroepidemiology of *H. pylori* in blood donors and the relationship between *H. pylori* infection and ABO/Rh blood groups, especially in Iran. Therefore, this study was designed to evaluate associations between ABO/Rh blood groups and anti-*H. pylori* and anti-CagA seropositivity in blood donors in Ahvaz city, southwest Iran. The study population consisted of mostly O+ donors, in agreement with previous reports from Iran and other countries.[14–16] Our study revealed anti-*H. pylori* IgG seropositivity in 77.2% of blood donors, higher than the result of Al-Balushi et al.[17] in Oman (62.4%). In another study by Yordanov et al.[18] in Bulgaria, an anti-*H. pylori* IgG seropositivity rate of 72.4% was reported, in agreement with our results. The high prevalence of *H. pylori* infections remains a serious challenge in developing countries because of their remoteness, poor socioeconomic conditions, and low standards of hygiene.[19]

In this study, we found that blood group O/Rh+ donors had higher seroprevalence of anti-*H. pylori* IgG antibodies compared with donors with other blood groups, but no statistically significant relationship was observed between seropositivity and any blood group. This finding was in line with reports by Mabeku et al.[20] in Cameroon and Iravani et al.[21] in Iran; both studies reported no associations between ABO/Rh blood groups and *H. pylori* seroprevalence. In another study, Aryana et al.[22] investigated associations between *H. pylori* and the Lewis and ABO blood systems in Iranian patients with dyspepsia. No significant associations were identified, in agreement with our results.

In contrast with the results of the current study, some reports have found that individuals with blood group O have higher seroprevalence of *H. pylori*. Gasim et al.[23] reported a positive association between the O blood group and *H. pylori* seropositivity among pregnant women in Sudan. Majeed et al.[24] observed significantly higher seroprevalence of *H. pylori* among patients with the O blood group suffering from gastroduodenal diseases in Erbil city, Iraq. This positive association may have occurred because the H antigen of the O blood group represents a receptor in gastric mucosal cells that enhances *H. pylori* colonization of the mucosal layers. Thus, individuals with blood group O may be more susceptible to infections caused by *H. pylori*.[25]

One potential explanation for discrepancies across prior studies of the associations between *H. pylori* and blood groups is the use of diverse techniques. Some researchers have used *H. pylori* fecal antigen tests, urease tests, and molecular methods to investigate such associations. In a study in Ethiopia, Teshome et al.[25] reported no association between ABO blood group and *H. pylori* in patients with peptic ulcers using stool antigen tests. However, Seid et al.[26] conducted a study in Ethiopia and found a positive link between O blood group and *H. pylori* in patients with upper abdominal disorders using fecal antigen screening. In another study by Mohammed et al.[27] in Sudan, the presence of *H. pylori* was investigated in gastric biopsies from patients with dyspeptic symptoms using the urease test. The authors found a positive correlation between the O/Rh+ blood group and *H. pylori* detection.

The seroprevalence of anti-CagA IgG was also investigated in the current study. The seroprevalence of anti-CagA IgG was 42.5% among blood donors, lower than that reported previously in a study in the center of Iran that found a 46.9% seroprevalence in healthy individuals.[28] Rahman et al.[29] also reported a lower seroprevalence of anti-CagA IgG (39.5%) in Bangladeshi patients with dyspepsia. In another study, Bonyadi et al.[30] reported a lower seroprevalence of anti-CagA IgG (35.6%) in patients with dyspepsia from the northwest of Iran. These inconsistencies may be relate to differences in the epidemiology of *H. pylori* infection in the regions being studied, the numbers of study participants, and the participants' underlying health status.

Some studies have concluded that higher serum anti-CagA IgG titers are significantly associated with gastric mucosal inflammation. Thus, this marker may be considered a risk factor for the development of gastric cancer.[31,32]

The current study showed that donors with blood group O/Rh+ had higher seroprevalence of anti-CagA IgG marker than donors of other blood groups. However, no significant associations were detected between anti-CagA IgG seropositivity and any blood groups. These results agreed with the finding of Afsharipour et al.[28] in Iran, who reported no statistically significant association between anti-CagA IgG seropositive and ABO/Rh blood groups in healthy individuals. Few studies have examined the association between anti-CagA

IgG seropositivity and blood groups, and thus limited data are available for comparison with our results.

In this study, we observed significant associations between anti-*H. pylori* IgG and anti-CagA IgG seropositivity and smoking, in agreement with previous studies.[33,34] Smoking may increase the risk of gastric cancer in *H. pylori*-seropositive individuals. The relationship between smoking and *H. pylori* seropositivity may be explained by the action of cigarette smoke carcinogens on the gastric mucosa, including oxidative stress and impairment of the gastric immune system that protects against *H. pylori* infection.[35] Nicotine reduces gastric mucosal blood flow, epidermal growth factor secretion, and mucus secretion, thus facilitating colonization of the mucosal layers by *H. pylori*.[35] However, this association was not observed in a study by Yordanov et al.[18] Lorenzo et al.[36] reported higher CagA seropositivity in non-smokers compared with smokers in Spain. One limitation of our study was related to age and sex. Very few women and older individuals are referred to transfusion centers for blood donation, and individuals younger than 18 years of age are unable to donate blood. Thus, we could not assess the influence of age and sex on associations between *H. pylori* seropositivity and blood groups in our study population. A second limitation of our study was its small sample size. A third limitation was the lack of endoscopic examination of blood donors. Because blood donation is completely voluntary, any interference in donors' lives may cause them to stop donating. The findings of our study indicated that *H. pylori* seropositivity was common in healthy blood donors. Ours represents the first study to analyze associations between anti-*H. pylori* and anti-CagA seropositivity and ABO/Rh blood groups among blood donors in southwest Iran. No significant associations were identified. Future studies should confirm our results by testing a larger number of participants of both sexes and with a wider age range.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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