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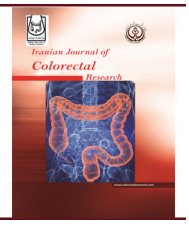

Effects of ABO Blood Groups and *Helicobacter pylori* on COVID-19 Susceptibility and Disease Severity: Findings from a Cross-Sectional Analysis

Maryam Nejabat*[1], *PhD;* *Mohammad Motamedifar*[2], *PhD; Saeid Amiri Zadeh Fard*[1*], *PhD

[1]*Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
[2]*Department of Bacteriology and Virology, Shiraz Medical School, Shiraz University Medical Science, Shiraz, Iran*

Corresponding authors:
Saeid Amiri Zadeh Fard, PhD; Gastroenterohepatology Research Center, Khalili st, Mohammad Rasoulallah Research Tower, Shiraz, Iran. **Tel:** +98 9393015217; **Email:** mrzdh_mr@yahoo.com

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Abstract

Background: The investigation of the correlation between ABO blood groups and susceptibility to infectious diseases, particularly COVID-19 and *Helicobacter pylori (H. pylori)*, has garnered significant scholarly attention in recent studies. This study aimed to evaluate the association between ABO blood groups and *H. pylori* infection with COVID-19 susceptibility and severity.

Methods: This cross-sectional study was conducted from October 1, 2021 to March 31, 2023. During this period, serum samples from 1,105 patients diagnosed with COVID-19 at hospitals affiliated with Shiraz University of Medical Sciences were analyzed for SARS-CoV-2 and *H. pylori* antibodies using the enzyme-linked immunosorbent assay (ELISA) technique. Clinical data, including demographic characteristics and manifestations of COVID-19, were collected through structured interviews and comprehensive reviews of medical records.

Results: The prevalence of COVID-19 was significantly higher among individuals with blood group A compared to other groups (P=0.032). Patients co-infected with *H. pylori* had more severe COVID-19 symptoms (P=0.021). The findings from this investigation reveal that blood group O is correlated with an increased risk for both COVID-19 and *H. pylori* infections, whereas blood group AB demonstrates a reduced susceptibility to COVID-19, although this does not imply definitive protective. Noteworthy associations were identified between ABO blood groups and specific COVID-19 symptoms, including headache, chronic underlying conditions, chest discomfort, and sore throat. The hospitalization among patients with blood group AB was higher than that observed in other blood groups (10.8%), while it was notably lower in patients with blood group O (4.6%, P=0.038).

Conclusion: Our findings suggest a significant association between ABO blood groups, *H. pylori* infection, and susceptibility to COVID-19. These factors may contribute to disease severity and warrant further investigation for potential clinical implications.

Keywords: ABO blood group; Helicobacter pylori; COVID-19; Susceptibility; ACE2 Receptor

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Introduction

Carbohydrates present on the surfaces of host cells play a crucial role in orchestrating interactions with microorganisms. These glycans function as ligands for pathogen lectins, thereby facilitating processes such as adhesion, invasion, and subsequent colonization. A prominent example of this phenomenon is the ABO blood group system, which is regulated by the ABO gene situated on chromosome 9q34.1-34.2 (1). ABO antigens are detectable on erythrocytes, leukocytes, platelets, and various tissues (2).

Contemporary research suggests that ABO blood groups may play a significant role in modulating susceptibility to various viral and bacterial infections. Accumulating evidence indicates a strong correlation between ABO blood groups and both susceptibility to and severity of COVID-19 (3). In particular, individuals with blood type O demonstrate a reduced risk of infection compared to those with blood type A (4). This correlation has been observed across various populations and remains statistically significant even after controlling for confounding variables such as age, sex, and comorbidities.

The correlation between ABO blood classifications and *Helicobacter pylori (H. pylori)* infection has attracted substantial scholarly interest in recent studies (5). De Mattos highlighted the structural heterogeneity of histo-blood group carbohydrates as potential receptors for *H. pylori* within the gastrointestinal system (6). The glycosyltransferases responsible for the biosynthesis of these carbohydrates are crucial for the bacterium's attachment to the gastric and duodenal mucosal surfaces. This adhesion is influenced by quantitative differences among histo-blood group phenotypes, which may affect vulnerability to *H. pylori*-associated pathologies. These discrepancies in the association between ABO blood groups and *H. pylori* infection may arise from several contributing factors. Genetic diversity among different populations can influence both the distribution of ABO blood types and the virulence of circulating *H. pylori* strains. Furthermore, methodological differences across studies—such as variations in diagnostic techniques, sample sizes, and demographic characteristics—may account for inconsistent findings. Environmental factors, including regional variations in hygiene, dietary habits, and socioeconomic status, could also play a role in modulating infection rates. Collectively, these elements suggest that the interplay between ABO phenotypes and *H. pylori* infection is complex and context-dependent.

Collectively, these investigations suggest that ABO blood types may play a significant role in mediating the association between *H. pylori* infection and gastrointestinal disorders, highlighting the necessity for further exploration to formulate potential management and preventive measures. Some studies have documented an elevated prevalence of *H. pylori* infection among individuals with blood type O (7-9), while others have noted a heightened incidence among those with blood type A (10-12). These observations underscore a complex interplay between ABO blood groups and *H. pylori* infection, suggesting that blood type may influence both susceptibility to infection and the host inflammatory response. Given the potential of *H. pylori* to upregulate ACE2 expression and exacerbate systemic inflammation, its interaction with ABO phenotypes may have broader implications for individual vulnerability to COVID-19 and its complications. Therefore, the aim of this study is to evaluate the independent and combined effects of ABO blood groups and *H. pylori* infection on susceptibility to COVID-19 and the severity of its clinical manifestations.

Methods and Materials

This cross-sectional study enrolled 1,105 patients with PCR-confirmed COVID-19 who were consecutively admitted to hospitals affiliated with Shiraz University of Medical Sciences from October 1, 2021 to March 31, 2023. Participants were included based on confirmed diagnoses and the availability of complete medical records. Individuals who declined to participate or had incomplete data were excluded from the analysis.

Venous blood samples were collected from all eligible participants to determine ABO blood group status and to assess the presence of antibodies against *H. pylori* and SARS-CoV-2. Antibody detection was performed using the enzyme-linked immunosorbent assay (ELISA) method, following standard protocols. The study protocol was reviewed and approved by the Research Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1400.089).

All samples were collected under standardized hospital conditions, typically during morning rounds, without the need for fasting. Blood samples preserved with Ethylene diamine tetra acetic acid (EDTA) were analyzed for blood grouping on the same day in the laboratory of the Gastroenterohepatology Research Center, following relevant guidelines. Serum was separated from clotted blood specimens and stored at -20°C immediately after collection. Although storage durations varied by patient, all samples were analyzed within three months to ensure consistency and minimize degradation.

Demographic characteristics, clinical symptoms related to COVID-19, and other relevant variables were documented using a standardized data collection form. To ensure a comprehensive clinical assessment, additional information—including symptom profiles, laboratory parameters, and radiological findings—was extracted from the patients' medical records. In accordance with ethical standards, written informed consent was obtained from all participants prior to blood sampling and data collection.

COVID-19 Detection

Antibody assays for COVID-19 were conducted on serum samples using the ELISA method (Monobind, UK). Both IgG and IgM antibodies specific to SARS-CoV-2 were measured. According to the manufacturer's guidelines, a titer greater than 11 RU/mL was considered positive. Patients with antibody levels exceeding this threshold for either IgG or IgM were classified as COVID-19 seropositive.

H. pylori Detection

Antibody assays for *H. pylori* were conducted on serum samples using the ELISA methodology (Monobind, UK). Patients demonstrating positive IgG and IgM antibody titers were considered to be infected with *H. pylori*.

Statistical Analysis

Data analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). The Chi-square statistic and P-value (<0.05) indicated a statistically significant association between ABO blood groups and *H. pylori* infection.

Results

SARS-CoV-2 Ab

ELISA evaluations were conducted on serum samples obtained from 515 female and 590 male participants to validate COVID-19 infection. A total of 479 (76.6%) women and 469 (79.5%) men were identified as having COVID-19 and were subsequently included in this research. The participants' ages ranged from 22 to 68 years (Table 1).

Blood Group Typing

The distribution of ABO blood typing among 948 COVID-19 patients indicated that blood type O was the most prevalent among those diagnosed with COVID-19, whereas blood type AB was the least prevalent (Figure 1).

The association between ABO blood groups and symptoms among 948 patients with COVID-19 was examined and is summarized in Table 1. A significant association was identified between ABO blood groups and the manifestation of specific clinical symptoms, including age, headache, and chronic underlying conditions in the context of COVID-19 infection.

Patients under the age of 40 with blood groups A and B exhibited a heightened susceptibility to COVID-19 infection (78.2% and 53.7% respectively), in contrast to blood groups O and AB (41.3% and 46.6% respectively, P=0.039). Regarding headaches, patients with blood group A reported headache symptoms significantly more frequently than individuals with other blood groups (59.5%, P<0.001).

On the contrary, the prevalence of sore throat was more pronounced in individuals with blood group AB (33.3%) and less prevalent in those with blood group A (15.4%), with a statistically significant difference (P=0.004).

Table 1: clinical manifestation in ABO blood groups [n (%)].

	A	B	O	AB	Total	Chi square	P value
Age <40	119(78.2)	115(53.7)	151(41.3)	56(46.6)	441	8.33	0.039
Age ≥40	132(21.8)	100(46.3)	211(58.6)	64(53.3)	507		
Male	110(43.6)	109(50.4)	193(53.1)	57(47.5)	469	5.52	0.137
Female	141(56.3)	106(49.5)	169(46.9)	63(52.5)	479		
Fever+	183(72.6)	145(67.59	233(64.1)	76(63.3)	637	5.67	0.128
Fever-	68(27.3)	70(32.4)	129(35.8)	44(36.6)	311		
Cough+	110(43.6)	104(47.7)	173(47.5)	62(51.6)	449	2.26	0.519
Cough-	141(56.3)	111(52.3)	189(52.5)	58(48.3)	499		
Sputum production+	23(9.1)	32(14.8)	34(8.6)	17(14.1)	106	7.5	0.057
Sputum production-	228(90.9)	183(85.1)	328(91.3)	103(85.8)	842		
Dyspnea+	70(27.3)	63(29.1)	83(22.5)	35(29.1)	251	4.26	0.234
Dyspnea-	181(72.6)	152(70.8)	279(77.5)	85(70.8)	697		
Myalgia or fatigue+	222(88.1)	182(84.2)	304(83.6)	99(82.5)	807	3.06	0.38
Myalgia or fatigue-	29(11.9)	33(15.7)	58(16.3)	21(17.5)	141		
Headache+	150(59.5)	81(37.5)	140(38.3)	61(50.8)	432	34.4	0.001
Headache-	101(40.4)	134(62.5)	222(61.6)	59(49.1)	516		
Sore throat+	40(15.4)	55(25.9)	98(27.2)	40(33.3)	233	17.79	0.004
Sore throat-	211(84.5)	160(74.1)	264(72.7)	80(66.6)	715		
Chronic underlying diseases+	196(78.1)	173(80.5)	199(54.7)	95(79.1)	663	64.21	0.001
Chronic underlying diseases-	55(21.8)	42(19.4)	163(45.2)	25(20.8)	285		
Chest pain+	32(13.1)	43(20.3)	37(9.7)	21(17.5)	133	14.1	0.002
Chest pain-	219(86.9)	172(79.6)	325(90.2)	99(82.5)	815		
Hospitalized+	22(8.3)	10(4.6)	10(2.7)	13(10.8)	55	15.3	0.001
Hospitalized-	229(91.6)	205(95.3)	352(97.2)	107(89.1)	893		
Other symptoms+	194(77.3)	157(73.1)	260(71.6)	98(81.6)	709	6.07	0.1
Other symptoms-	57(22.6)	58(26.8)	102(28.3)	22(18.3)	239		

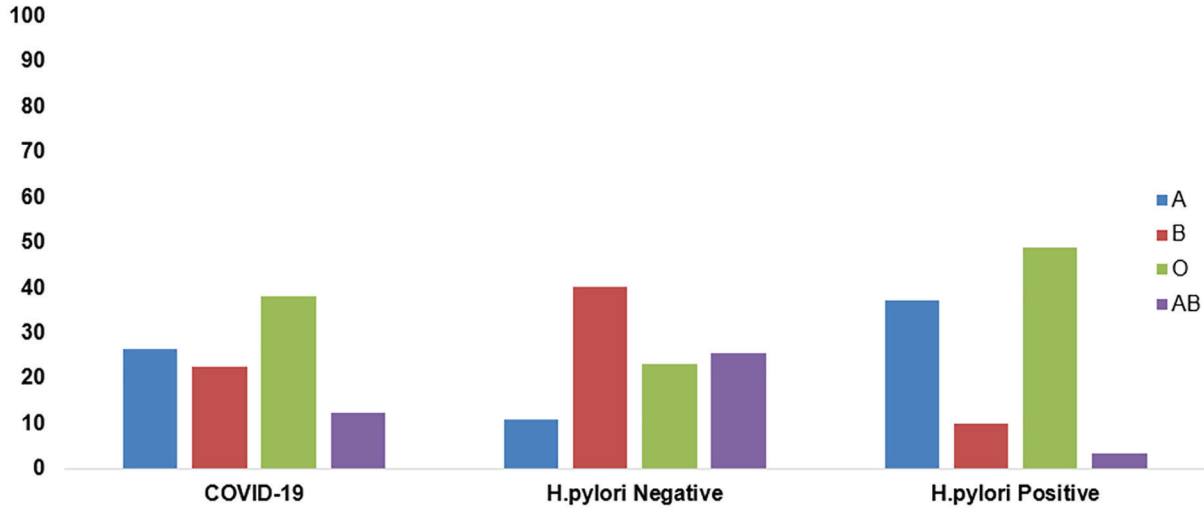

Figure 1: The rate of infection with Helicobacter pylori and covid-19 in blood groups (percentage)

Table 2: The relationship between Helicobacter pylori infection and Covid-19 infection in different blood groups [n (%)].

	A	B	O	AB	Total	Chi-square	P value
HP+	20(21.9)	57(6.0)	271(28.6)	19(2.0)	555(58.54)	281.1	0.001
HP-	43(4.5)	158(16.7)	91(9.6)	101(10.6)	393(41.45)		

HP+: H. pylori infection positive; HP-: H. pylori infection negative

The presence of chronic underlying conditions and chest pain among patients with blood group O demonstrated a lower impact (54.7%, P<0.001 and 9.7%, P=0.002 respectively), in contrast to patients with blood group B, who exhibited a higher susceptibility to COVID-19 infection compared to other blood groups (80.5%, P=0.001 and 20.3% respectively, P=0.002).

With regard to other symptoms and gender, there was no significant correlation between fever, cough, sputum production, dyspnea, myalgia or fatigue, and the manifestation of other symptoms across all blood groups.

The incidence of hospitalization among patients with blood group AB was higher than that observed in other blood groups, while it was significantly lower in patients with blood group O (10.8% and 2.7%, respectively; P=0.001).

H. pylori Ab

ELISA evaluations for *H. pylori* antibodies were performed on serum samples from 948 COVID-19 patients enrolled in this study. The quantity of positive *H. pylori* cases is detailed in Table 2.

208 individuals with blood group A (82.53%), 58 individuals with blood group B (26.85%), 271 individuals with blood group O (70.03%), and 39 individuals with blood group AB (65%) tested positive for *H. pylori* antibodies.

In terms of assessing the influence of ABO blood groups on *H. pylori* infection, the highest prevalence of *H. pylori* infection was found in individuals with blood group O, at 28.6%, compared to just 2.0% in those with blood group AB (P=0.001; Table 2).

Discussion

Our study demonstrated a statistically significant association between blood group A and increased susceptibility to and severity of COVID-19, particularly in patients co-infectedwith *H. pylori* within a large cohort of Iranian patients. The findings confirm and extend previous studies suggesting that ABO blood phenotypes play a role in modulating susceptibility to SARS-CoV-2 infection. While certain studies have reported no association between ABO blood type and the severity or mortality associated with COVID-19 infections (13-15), other research has indicated an elevated risk of COVID-19 infection in individuals with blood group O (4, 16). Our findings exhibit partial alignment with these investigations, underscoring the complex nature of this relationship. This observation is consistent with the hypothesis that anti-A and anti-B antibodies present in individuals with non-AB blood types may provide a degree of protection against the virus. However, our study does not demonstrate a definitive protective effect for blood type O, as noted in the works of Zhang et al. and Ray et al. (4, 14) Comprehensive investigations are imperative to resolve these inconsistencies. Factors such as geographical discrepancies in viral strains and pre-existing health conditions within diverse study populations require further exploration.

We identified associations between blood type and clinical manifestations, including headache, chronic underlying diseases, chest pain, and sore throat. These results necessitate further investigation to clarify the underlying biological mechanisms. It is conceivable that ABO blood groups may modulate the immune

response or influence viral entry pathways, resulting in variations in symptomatology. This study also implies a relationship between blood type AB and an increased likelihood of hospitalization. Our findings are consistent with those of Kusumoto et al., who documented a heightened risk of severe cases in COVID-19 patients with blood type AB (17). Further studies are essential to validate this association and to investigate the potential underlying causes.

Almorish et al. demonstrated that ABO blood groups exhibited significant differences between males and females infected with COVID-19; however, we did not observe a significant correlation between the sex of these patients and their blood group type (18). Multiple pathophysiological mechanisms have been proposed to clarify the association between ABO blood type and SARS-CoV-2 infection. Anti-A and/or anti-B antibodies may bind to A and/or B antigens expressed on the viral envelope, thereby obstructing the infection of target cells (19). In essence, these naturally occurring antibodies could act as neutralizing agents. The seemingly protective effect of blood type O against COVID-19, as indicated in certain studies, has been attributed to the antagonistic action of anti-A antibodies on the interaction between SARS-CoV-2 and ACE-2 receptors (10-12). Moreover, similar to our study, these studies indicated that blood group O was predominant among patients infected with *H. pylori*, particularly among secretors, and a significant association between ABO blood groups and *H. pylori* infection was observed. Zakaria Chakrani demonstrated that individuals with blood group O were more susceptible to *H. pylori* infection, while those with blood groups B and AB exhibited a lower likelihood of such infections. The findings from this meta-analysis of observational studies suggest an estimated increased odds of *H. pylori* infection among individuals with blood group O (5).

On the contrary, Mohammadzadeh et al. demonstrated that there is no statistically significant correlation between blood group type and the incidence of *H. pylori* infection. Nevertheless, the incidence of blood type A was markedly elevated among individuals exhibiting a positive H. pylori IgG test (20).

Our findings further suggest an association between ABO blood groups and *H. pylori* infection; however, they do not support the claims made by Zakaria Chakarani, which indicate a potential protective effect of anti-A antibodies against this particular infection (5). Numerous investigations have indicated that individuals with blood type A exhibit a heightened risk of infection or increased severity of conditions compared to those with blood type O (7, 21, 22) . Nonetheless, we contest the assertion that this protective effect can be attributed to a singular underlying mechanism. Our observations suggest that blood type O, rather than blood type B, confers a degree of protection against COVID-19, despite the presence of anti-A antibodies in both blood types.

Recent evidence suggests that H. pylori, known for its long-standing interaction with the host immune system, can modulate mucosal immunity beyond the gastric environment. This modulation may influence inflammatory pathways in extra-gastric sites, including the respiratory tract, through systemic immune effects and low-grade inflammation (23-25). In this investigation, we also established that individuals with *H. pylori* infection have a significantly greater likelihood of contracting COVID-19 compared to their healthy counterparts. Such colonization may lead to conditions such as sinusitis and pharyngitis, while gastric aspiration could further exacerbate pulmonary inflammation. The presence of *H. pylori* within the gastrointestinal tract is of considerable significance, as it induces a chronic inflammatory state within the gastric mucosa. This ongoing inflammation may enhance the organism's response to infections, including SARS-CoV-2, consequently leading to more severe clinical outcomes for patients affected by COVID-19.

Furthermore, *H. pylori* infection can lead to the loss of parietal cells and an increase in intra-gastric pH (26). This change indicates that the normally low pH of gastric acid, which generally serves to inactivate viruses, becomes ineffective, allowing SARS-CoV-2 to persist for an extended duration within the gastrointestinal tract. As a result, *H. pylori* infection may exacerbate the severity of COVID-19 through mechanisms involving chronic inflammation, endothelial dysfunction, modified gastric conditions, and the presence of comorbidities (27). Each of these factors can further intensify the organism's response to SARS-CoV-2, highlighting the complex relationship between *H. pylori* infection and the severity of COVID-19.

This investigation is subject to several limitations. The retrospective design inherently includes the potential for confounding variables. In addition, our study population may not accurately reflect the broader population of Fars Province regarding the distribution of ABO blood groups. In Fars Province, the estimated prevalence of ABO blood groups is 25.7% for blood group A (38,503 individuals), 22.48% for group B (33,676 individuals), 39.15% for group O (58,648 individuals), and 12.65% for group AB (18,950 individuals). This regional distribution provides a contextual baseline for interpreting the overrepresentation or underrepresentation of specific blood groups in our study cohort. Given the heterogeneity in ABO blood group distribution across various populations, along with the observed associations between COVID-19 and *H. pylori* infection, it is imperative to conduct studies within more heterogeneous and ethnically diverse cohorts. Future research should prioritize replicating these findings in regions characterized by distinct demographic profiles, genetic variations, and environmental exposures. Such an approach will

facilitate the determination of whether the observed associations remain consistent on a global scale or exhibit significant variability across different population groups.

Conclusion

This study reveals a heightened prevalence of COVID-19 infections among individuals infected with *H. pylori*, while concurrently highlighting a significant association between ABO blood types and susceptibility to both COVID-19 and *H. pylori* infections. Individuals with blood group O exhibited an elevated risk for these infections, whereas those with blood group AB manifested a reduced susceptibility to COVID-19 and *H. pylori*; however, this does not imply a definitive protective effect. The relationship between *H. pylori* and COVID-19 is complex, involving various biological mechanisms that may affect disease progression. Understanding these interactions is imperative for formulating effective management protocols for patients affected by both conditions. The study emphasizes the complexity of the association between ABO blood types and infectious diseases, indicating that further research involving larger and more heterogeneous populations is essential to clarify the fundamental mechanisms and to formulate effective prevention and management strategies.

Authors' Contribution

Critical revision of the manuscript: Saeid Amiri zadeh fard; study concept and design: Maryam Nejabat; data acquisition, analysis and interpretation of data: Saeid Amiri zadeh fard, Maryam Nejabat; drafting of the manuscript: Mohammad Motamedifar; supervision of study processes: Mohammad Motamedifar. All the authors contributed to the final revision of this manuscript.

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Conflicts of interest: None declared.

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