

## The Novel Coronavirus SARS-CoV-2 Vulnerability Association with ABO/Rh Blood Types

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### ABSTRACT

**Background & Objective:** Coronavirus disease 2019 (COVID-19) is the most recent emerging viral disease. Defining the epidemiological aspects and factors influencing the susceptibility of the patients to COVID-19 has been an ongoing struggle. In the present study, we have investigated the connection between ABO histo-blood group phenotypes and the COVID-19.

**Methods:** This study was conducted on 397 patients with confirmed diagnoses of COVID-19 admitted to our center. Also, 500 individuals were selected to form the controls, all of whom had been disclosed to the same medical center in June 2019, before the onset of the outbreak.

**Results:** Our results demonstrated ABO histo-blood phenotypes are correlated with patients' susceptibility to the infection. A higher rate of infection was observed among patients with the AB histo-blood group, while patients with the O histo-blood group have shown a lower rate of infection. The Rh blood group phenotype was not statistically significant in determining a patient's vulnerability.

**Conclusion:** Similar to several previous studies about other viral diseases' association with ABO histo-blood groups, we have concluded that an individual's ABO histo-blood group phenotype and his/her susceptibility to COVID-19 are indeed connected. So far, only one research has been conducted about this association. Interestingly, while we observed a decreased vulnerability to the disease among patients with an O histo-blood group, we have reached discordant results regarding the increased susceptibility among individuals with an AB histo-blood group, unlike A histo-blood group in the previous study.

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### Introduction

Coronaviruses have been the cause of three recent global outbreaks, Severe acute respiratory syndrome (SARS, 2002 to 2003), Middle East respiratory syndrome (MERS, since 2012), and most recently, the coronavirus disease (COVID-19) posing an extensive crisis in late 2019 caused by Severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2), formerly recognized as 2019-nCoV (1-3). Although the aforementioned coronaviruses, have caused fatal diseases in humans, other known coronaviruses have only induced mild flu-like symptoms in healthy individuals (4).

COVID-19 patients mostly present with fever, dry cough, and dyspnea, which can progress to acute respiratory distress syndrome and multiple organ failures (5).

While most patients have been considered to have a good prognosis, multiple studies demonstrated that older male adults and patients with pre-existing medical conditions such as underlying respiratory, cardiovascular, and immunodeficiency diseases are more susceptible to poor clinical outcomes due to their reduced immune system function (6,7).

Other innate and adaptive factors can affect viral infection susceptibility or resistance. Previous researches have proved the potential role of ABO blood groups on a host's genetic susceptibility to various viral diseases such as influenza, Ebola, enteric viruses, and SARS-CoV infections (8-12).

This association could be on account of ABO-antibodies, which act as a part of innate immunity

against some bacterial and viral agents that bear ABO-antigens (13).

We conducted this study to further investigate the susceptibility variabilities amongst patients with different ABO histo-blood groups. Knowledge of innate susceptibilities to COVID-19 disease could lead to a better understanding of virus pathogenesis, disease management, and patient survival.

## Materials and Methods

In this cross-sectional survey, individuals with diagnoses of COVID-19 admitted to Imam Khomeini Hospital Complex, Tehran, Iran, during March 2020 were enrolled in the study. We included 397 patients with positive real-time polymerase chain reaction (RT-PCR) test results performed on swabs with synthetic fibers from both nasopharyngeal and oropharyngeal samples. The diagnoses were established by utilizing Novel Coronavirus (2019-nCoV) Real-Time Multiplex RT-PCR kit (Daan Gene Co. Ltd.) and Novel Wuhan CoV E-gene kit (TIB Molbiol, Germany) according to manufacturer's instructions on CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., USA). Demographic characteristics were collected from each patient's file. Subjects' ABO and Rh blood group determination was conducted using the tube method forward type and back type grouping techniques. The forward grouping was performed on a 3% red blood cell (RBC) suspension using commercially prepared monoclonal blood group antisera to check antigens on the surface of RBC provided by CE-Immundiagnostika GmbH, Germany. In reverse grouping, the patients' plasma was tested against commercially prepared reagent cells of known A1 and B phenotype to determine the anticipated ABO antibodies in the patients' serum using red cell reagents provided by DIAMED GmbH, Switzerland. ABO and Rh blood grouping data retrieved from the Imam Khomeini hospital's blood bank database of patients referred in June 2019 to our lab, before the onset of the outbreak, were used as subjects uninfected by COVID-19 (control). COVID-19 negative blood samples referred from outpatient and inpatient services constitute 136 and 364 of specimens, respectively. Outpatient samples referred from specialist clinic of the hospital (Dr Yalda clinic, Tehran, Iran) and inpatient samples referred from accident and emergency department (n=103), liver transplant unit (n=54), plastic surgery unit (n=53), otolaryngology unit (n=46), neurosurgery unit (n=39), urology unit (n=35), and orthopedic unit (n=34).

The research protocol was confirmed by the Ethics Committee of Tehran University of Medical Sciences (code: IR.TUMS.VCR.REC.1399.208). All data were entered and analyzed using IBM SPSS 26 (SPSS Inc., Chicago, IL, USA). Comparisons of proportions of blood group antigens between COVID-19 patients and controls were conducted using Pearson's Chi-square test ( $\chi^2$ ) or Fisher's exact test where appropriate. We explored the combined effect of various blood groups on COVID-19 susceptibility using binary logistic regression analysis. Only histo-blood groups AB and O with a statistically

significant association with COVID-19 status in bivariate analyses were included in the model (multivariate analysis for adjustment of age and gender variables), and the results were reported as odds ratio with 95% confidence interval. We used an independent-sample T-test to compare the means. P-values of less than or equal to 0.05 were considered statistically significant.

## Results

In the present study, 397 COVID-19 patients and 500 normal controls were analyzed to evaluate the association of the ABO histo-blood group phenotypes with COVID-19 disease in the Iranian population. Patients' ages ranged from 15 to 95 years old versus controls' ages reached 4 to 93 years old. Patients and controls had a mean (SD) age of 58.81 (15.4) and 48.53 (17.9) years, respectively, which showed that the older population is at a higher risk of infection with SARS-CoV-2 ( $P<0.001$ ). Mean (SD) age of patients admitted to the intensive care unit (ICU) and general wards were 62.64 (13.9) and 57 (15.8), respectively, which showed that older patients are more prone to the more severe complications and requiring ICU admission ( $P<0.001$ ).

Our study demonstrated that men were infected more than women by coronavirus so that among patients and controls, male-to-female ratios were 1.74:1 (252 males and 145 females) and 0.86:1 (231 males and 269 females), respectively ( $P<0.001$ ). Among the patients, 270 (68%) individuals were admitted to the ICU, among whom 86 (67.7%) and 41 (32.3%) were males and females, respectively. Furthermore, 127 (32%) individuals were admitted to the general wards, among whom 166 (61.5%) and 104 (38.5%) were males and females, respectively. There was no statistically significant difference in the severity of disease with respect to genders ( $P=0.23$ ).

The percentages of A, B, AB, and O histo-blood groups in the patients were 40.3%, 22.4%, 9.3%, and 28%, whereas, in the controls, these values were 36%, 21%, 5%, and 38%, respectively. When the blood groups of the COVID-19 patients were compared with the controls, patients with AB histo-blood group (OR=2.02; 95%CI: 1.17-3.51) and O histo-blood group (OR=0.68; 95%CI: 0.5-0.92) were found to have significantly higher and lower proportions than controls, respectively in univariate and multivariate logistic regressions (Table 1). Our study has suggested that O histo-blood group makes individuals less susceptible to SARS-CoV-2 virus infection, unlike the AB histo-blood group that has the opposite effect. As shown in Table 1, there was no statistically significant association between COVID-19 positivity and Rh blood group ( $P=0.66$ ). The percentage of histo-blood groups A, B, AB, and O in the ICU admitted patients were 40.2%, 22%, 7.9%, and 29.9%, whereas, in the mild patients, these values were 40.4%, 22.6%, 10%, and 27%, respectively (Table 2). As shown in Table 2, there was no association between the severity of COVID-19 and ABO histo-blood group phenotypes and Rh blood groups ( $P=0.88$ ,  $P=0.32$ , respectively).

**Table 1.** Comparison between patients and controls with regards to ABO/Rh blood group

		Controls (n=500)	Patients (n=397)	OR (95% CI)		P-value
				Crude	Adjusted	
<b>Age, mean (SD)</b>		48.53 (17.9)	58.81 (15.4)	-	-	
<b>Sex</b>	Male	231 (46.2%)	252 (63.5%)	-	-	
	Female	269 (53.8%)	145 (36.5%)	-	-	
<b>Blood groups, n(%)</b>	Rh negative	55 (11%)	40 (10.1%)	0.91 (0.59-1.39)	0.91 (0.58-1.43)	0.66
	Rh positive	445 (89%)	357 (89.9%)	1 (referent group)	1 (referent group)	
	A	180 (36%)	160 (40.3%)	1.2 (0.92-1.57)	1.16 (0.87-1.55)	0.19
	B	105 (21%)	89 (22.4%)	1.09 (0.79-1.5)	1.01 (0.72-1.42)	0.61
	AB	25 (5%)	37 (9.3%)	1.95 (1.16-3.3)	2.02 (1.17-3.51)	0.01
	O	190 (38%)	111 (28%)	0.63 (0.48-0.84)	0.68 (0.5-0.92)	0.002

**Table 2.** Comparison of disease severity among covid-19 patients with different blood groups

		ICU (n=127)	General wards (n=270)	OR (95% CI)		P-value
				Crude	Adjusted	
<b>Age (year), mean (SD)</b>		62.64 (13.9%)	57 (15.8%)	-	-	<0.001
<b>Sex</b>	Male	86 (67.7%)	41 (32.3%)	-	-	0.23
	Female	166 (61.5%)	104 (38.5%)	-	-	
<b>Blood groups</b>	Rh negative	10 (7.9%)	30 (11.1%)	0.68 (0.32-1.45)	0.7 (0.33-1.49)	0.32
	Rh positive	117 (92.1%)	240 (88.9%)	1 (referent group)	1 (referent group)	
	A	51 (40.2%)	109 (40.4%)	0.99 (0.65-1.52)	0.96 (0.62-1.48)	0.97
	B	28 (22%)	61 (22.6%)	0.97 (0.58-1.61)	0.98 (0.59-1.64)	0.9
	AB	10 (7.9%)	27 (10%)	0.77 (0.36-1.64)	0.8 (0.37-1.72)	0.5
	O	38 (29.9%)	73 (27%)	1.15 (0.72-1.84)	1.17 (0.73-1.89)	0.55

## Discussion

Viral diseases are considered massive sources of worldwide mortality and morbidity and have significant potential impacts on the global economy and human health. They are regarded as the cause of 60% and 75% of currently known and newly-appearing infectious diseases, respectively (14,15).

The COVID-19 is the latest fatal zoonotic disease, and since its advent, it has affected thousands of people throughout the world. As yet, multiple risk factors, including older age, male gender, and presence of chronic underlying comorbidities, have been established to be attributed to the higher probability of getting infected with SARS-Cov-2 (6,7). Concordantly, we

found that the COVID-19 affects older individuals to a greater extent so that the mean age of patients was about ten years higher than uninfected individuals. Also, according to our survey, the male gender demonstrated as a risk factor in cases of SARS-CoV-2 infection, too, with the male-to-female ratios of 1.74:1 among patients and 0.86:1 among controls which demonstrated a statistically significant difference.

Numerous efforts have been made to identify factors that have an impact on the susceptibility of infected patients. While a connection between ABO/RH blood groups and other viral disease susceptibility has been

demonstrated previously, there is not much information about this connection regarding SARS-CoV-2.

ABO histo-blood group system is composed of three antigens—A, B and H. Sequential addition of carbohydrate units on precursor oligosaccharide backbone leads to four phenotypes A, B, AB and O; H antigen is built by the addition of fucose to the oligosaccharide backbone; moreover, A and B antigens are made up of addition of N-acetylgalactosamine (A) and D-galactose (B) to the core H antigen, respectively (13,16-18). RH blood grouping categorizes in two phenotypes D+ and D- depending on the presence or absence of D protein epitopes (16-18). ABO histo-blood group antigens have been found in RBC's surface lymphocytes, as well as many tissue organs, mucosal surfaces, and exocrine secretions (13,19-23). The human body produces antibodies against the missing ABO histo-blood group antigens resulting in the production of anti-A and/or anti-B antibodies (13,24).

ABO histo-blood groups are genetically inherited traits that are distributed variably among different populations, O and AB blood type have the most and least prevalence in many populations (16-19). In our study, the percentages of A, B, AB, and O histo-blood groups in uninfected individuals who were employed as controls were 36%, 21%, 5%, and 38%, respectively.

Our study found that the vulnerability to the COVID-19 infection was higher among Iranians with an AB histo-blood group and lower in those with an O histo-blood group. A study conducted by Jiao Zhao *et al.* in China has similarly demonstrated that Chinese patients with O histo-blood group are less likely to suffer from a COVID-19 infection. On the other hand, according to the Chinese study, individuals with A histo-blood group were identified as high-risk. Different patterns of the outbreak in Iran and China might be due to this disparity given the fact that AB is the least prevalent histo-blood group among populations and the number of individuals with an A histo-blood group is generally higher; as a result, it could be suggested that less percentage of Iranians are susceptible to COVID -19 comparing to China. However, limitations of these studies and the biologic difference between these two populations should not be overlooked (25).

Previous studies propose a mechanism through which ABO histo-blood groups interact with viruses. ABO histo-blood antigens have an impact on the immune system and affect pathogens spread by means of the host's natural antibodies and complement systems (13,19,20). Multiple studies have been conducted about the relationship between various viruses' biological functions and ABO histo-blood groups leading to human host viral disease vulnerability or resistance. It has been suggested that some viruses perform their role by binding to ABO histo-blood antigens. Norwalk-like viruses and bat caliciviruses spread through interaction with ABO histo-blood group antigens (1,2). The host's histo-blood group antigens affected human Rotavirus susceptibility and reduced vaccine efficacy (23).

The role of ABO histo-blood group phenotype on the probability of getting infected with SARS-CoV, the causative agent of the severe acute respiratory syndrome (SARS), is presumed. SARS-CoV invades respiratory and GI tract mucosal epithelium where the epithelial cells express ABO histo-blood group antigens, through interaction between virus spike proteins and receptor angiotensin-converting enzyme 2 (ACE2). In one study, Yufeng Cheng *et al.* investigated the prevalence of SARS disease among forty-five health-care workers who had unprotected exposure to infected patients, finding that individuals with histo-blood group O had a lower likelihood of infection, allegedly because of SARS-CoV varying binding capacity in different blood group types (10). Patrice Guillon *et al.* used a mathematical cellular viral transmission model. They claimed that this association could be attributed to the protective role of anti-histo-blood group antibodies preventing the virus from adhesion to its receptor on the host cells (26). Considering that the SARS-Cov and SARS-CoV-2 viruses are genetically related to each other, and the protective pattern of the O histo-blood group in both viruses is similar, the abovementioned rationalization could be extended to SARS-CoV-2 as well. Further studies are required to determine the exact mechanism through which ABO blood group influences COVID-19 susceptibility, which could be helpful in patient management and disease control.

## Conclusion

As demonstrated by previous Chinese research on COVID-19 and our current study, the statistically significant association of ABO histo-blood group with COVID-19 susceptibility is clear. However, our results were discordant regarding the ABO histo-blood antigens which make people susceptible to COVID-19 (AB versus A histo-blood group phenotype in Iran and China, respectively). We assume that since the AB histo-blood group constitutes the least prevalent proportion, and A histo-blood group is the second most prevalent blood group among the general population, the observed discrepancy between the findings of these two studies may help explain different epidemiological patterns among various ethnicities. More studies are needed to further investigate precise viral pathogenesis to explain such differences.

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## Conflict of Interest

The authors confirm that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

## Ethical statements

The proposal of the present study was reviewed and approved by “Ethics Organizing Committee in Tehran University of Medical Sciences (code: IR.TUMS.VCR.REC.1399.208)

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