ASSOCIATION OF COVID-19 WITH ABO BLOOD GROUPS IN TERTIARY CARE CENTER OF PAKISTAN

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ABSTRACT

Objective: To determine the relationship between ABO blood groups and the COVID-19 susceptibility.
Study Design: Cross-sectional analytical study.
Place and Duration of the Study: Department of Pathology, Army Medical College, in collaboration with Pak Emirates Military Hospital, Rawalpindi, from Mar to May 2020.
Methodology: The sample comprised of 326 patients. Non-purposive consecutive sampling technique was used with inclusion criteria of COVID-19 patients confirmed by real-time reverse transcriptase polymerase-chain-reaction test (RT-PCR) of sub acute respiratory syndrome corona virus-2 (SARS-CoV-2) of all age groups. ABO blood grouping was done by test tube method. The results were compared with recent surveys of ABO blood group distribution in general population of Pakistan.
Results: The ABO blood group distribution of normal population was compared with COVID-19 patients. The blood group A was found to be 122 (37.4%) having significant association with COVID infection and mortality but blood group O had least prevalence 71 (21.8%) with COVID-19 infection. The *p*-value was calculated using Chi-square test which was p=0.04.
Conclusion: Blood group A had a significant association where as people with blood group O had a least association for COVID-19 infection and severity. The findings of the present study can be used in clinical management of the COVID-19 and that blood group A population is more susceptible to acquire COVID-19 infection, further more can develop complications with high mortality rate. Clinicians should be more vigilant in treating patients with blood group A.

Keywords: ABO blood groups, Corona virus disease-19, Sub acute respiratory syndrome-corona virus-2.

INTRODUCTION

COVID-19 disease is a new infectious disease caused by a SARS-CoV-2, which is a zoonotic virus. In the past two decades, similar viruses have caused epidemics such as the Severe Acute Respiratory Syndrome Corona virus (SARS-CoV) in 2002, H1N1 influenza (bird flu) in 2009 and the Middle East Respiratory Syndrome Coronavirus (MERS-COV) in 2012. On December 31st 2019, pneumonia of unknown cause was detected in Wuhan city and reported to WHO country office in China. Since then it spread rapidly around the world. The World Health Organization confirmed the epidemic to be a Public Health Emergency of International Concern on 30th January 2020, and recognised it as a pandemic on 11th of March 2020. As per today May 2020, more than 4179,479 COVID-19 cases have been testified around the world, resulting in more than 287,525 deaths until now reported[1].

Until now in Pakistan total confirmed cases of COVID-19 are 35,298 with 759 deaths and 8,899 recovered cases[2]. Patient's old age, male gender and certain chronic medical diseases are risk factors for the infection of SARS-CoV-2 morbidity and mortality[3]. Currently there is no biological marker known for susceptibility to COVID-19. Two studies conducted recently in China and United States of America. One study was done in Wuhan and Shenzhen cities of China while the other study conducted in New York (University of Columbia) showed strong linkage between ABO blood groups and infection. They compared the results COVID-19 hospitalized patients with

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the percentages of the normal distribution of populations of cities of China (Wuhan and Shenzhen) and United States (New York). The studies result showed that frequency of blood groups A was higher and blood group O was lower in COVID-19 patients relative to the general population[4,5]. On the surface of human cells, carbohydrate epitopes are present according to the Landsteiner's ABO blood types. The contributing factors of A and B blood antigens are trisaccharide moieties Gal NAcα 1-3 (Fucα-1,2) Gal-β and Galα1-3 (Fucα-1,2) Gal-β, whereas Fucα-1,2 Gal-β are antigens of blood group O. ABO Blood groups are inherited genetically. The effect of environmental factors can actually cause which blood types in a people will be delivered on more recurrently to the next cohort. A number of diseases reported to have shown a strong association with the ABO blood systems. The presence or absence of these tissue antigens is more likely related throughout the body and not directly or primarily related to their presence on RBCs[6]. Large number of early researches done to associate the blood groups with number of diseases of infections, malignancy, and coagulation, counts on using statistical ways to narrate it. Broad researches with given scientific validations have shown to have its association in infectious disease, tumor immunology and membrane chemistry[7]. Previous studies had linked relationship of blood groups with a number of different diseases and infections. Infections of SARS-Co V-1 with type A8, H. Pylori with type O[9], Norwalk virus with type O blood group[10].

Blood group antigens association with COVID-19 has not been done in our population until now therefore this study was under taken. Present study was planned to analyze the incidence and association of ABO blood types distribution in COVID-19 patients. This data can be useful to the physicians to identify the high risk blood type in predicting the disease progression.

METHODOLOGY

This was a cross-sectional analytical study carried out in the department of Pathology, Army Medical College, in collaboration with Pak Emirates Military Hospital, Pakistan from March to May 2020 after approval of Ethics Review Committee of the Army Medical College, Rawalpindi with ERC number ERC/ID/27. Patients of all age groups irrespective of their gender were included after informed consent. The confidentiality of the patients was preserved and patients were coded. Non-purposive consecutive sampling technique was used with inclusion criteria of COVID-19 patients confirmed by real-time reverse transcriptase polymerase-chain-reaction test (RT-PCR) of sub acute respiratory syndrome corona virus-2 (SARS-CoV-2) of all age groups. According to the WHO latest statistics of COVID-19 the patients count in Pakistan is 148921 which make the incidence of the disease 0.07%, using this sample size and confidence interval to be 95% and error to be 5%. The minimum sample size to be is 100 using the WHO calculator. We enrolled a total of 326 patients of all age groups irrespective of their gender were included after informed consent. Performa was designed to endorse demographic data of age and gender, clinical signs and symptoms, and ABO, Rh blood grouping. The infected patients with COVID-19 blood samples were collected in pathology Laboratory of AM College. ABO grouping was done by tube method, with reverse and forward grouping method. A 2ml blood of the COVID-19 patients received in plain bottle for forward and reverse methods. In forward method patient's saline-washed red cells was mixed with known commercially prepared antiserum of anti-A, anti-B and anti-D in a test tube; the mixture was incubated at room temperature followed by centrifugation. For reverse method, patient's serum was mixed with reagent red cells of known groups of A, B and O (available commercially), incubated at room temperature followed by centrifugation. Then a red cell button observed at the bottom of the tube was examined for agglutination. Our results were compared with the latest studies of ABO blood groups among 2327 normal population of Pakistan. The statistical analysis was done using SPSS 20. Results were calculated using

mean for qualitative variables and percentage and frequencies for categorical variables. The *p*-value was calculated by using Chi-square test.

RESULTS

Our study had an inclusion of 326 patients of COVID-19. The mean of age calculated was 41.18 ± 12.56 years. The minimum and maximum range of age lies between 20-76 years. Out of 326 patients male predominance of 310 (95%) and only 16 (4.9%) were females. The male to female ratio was 19:1.

A total 326 COVID-19 infected patients in our study showed a blood group distribution of A-122 (37.4%), B-107 (32.8%), AB-26 (8.0%) and O-71 (21.8%). Blood group B has a high prevalence in our population followed by O, A and AB. As in table-I the blood group A proportion was with a highest number of COVID-19 patients that is 122 (37.4%) than in normal population distribution being 527(22.7%). The blood type O percentage distribution of COVID-19 infection was 71 (21.8%) significantly lower than that in normal population, being 619 (26.6%). The *p*-value was calculated by using Chi-square test which was significant, *p*-value 0.04. These results calculated showed that A blood type had a significantly increased risk for COVID-19 disease whereas O blood type had a decreased in risk for COVID-19 disease, in comparison with the groups. The Rh grouping showed 303 (92.9%) Rh positive while only 23 (7.1%) were Rh negative cases.

Out of 326 patients 128 were critically ill, further distribution shared blood group A is the most common group with frequency of 75 (61.5%) as per table-II. *p*-value calculated by using Chi-Square test which was found to be significant *p*-value <0.001.

The outcome of COVID-19 critical patients also shows a strong correlation with A blood group and negative association with O blood group. The percentages of blood group A in critically ill patients who were under treatment, on ventilators or those who expired were 44 (51.2%), 13 (61.9%) and 4 (50%) respectively with higher numbers of frequencies while the percentage of blood group O in the above mentioned patients showed 15 (17.4)%, 1 (4.8%) and 1 (12.5%) respectively with a less number of frequencies.

Table-I: Comparison of ABO blood group distribution in general population of Pakistan and COVID-19 positive patients of Pakistan.

Blood Group	Pakistan General Population[15]	Corona Positive	*p*-value
	Frequency (%)	Frequency (%)	
A	527 (22.7%)	122 (37.4%)	0.04
B	945 (40.6%)	107 (32.8%)	
AB	236 (10.1%)	26 (8.0%)	
O	619 (26.6%)	71 (21.8%)	

Table-II: Frequencies of blood groups in critical and non-critical patients.

Blood Group	Critical Yes	Critical No	*p*-value
	Frequency (%)	Frequency (%)	
A	75 (58.6%)	47 (23.7%)	<0.001
B	27 (21.1%)	80 (40.4%)	
AB	7 (5.5%)	19 (9.6%)	
O	19 (14.8%)	52 (26.3%)	

Association of mortality in COVID-19 positive patients indicated that patients with A blood group were at high risk and O blood group patients were at low risk was also observed. The proportions of ABO blood groups among the 08 expired patients were A-4 (50.0%), B-2 (25.0%), AB-1 (12.5%) and O-1 (12.5%) respectively as shown in figure. The blood group types were found to be significantly related with *p*-value of 0.04 with COVID-19 severity and mortality.

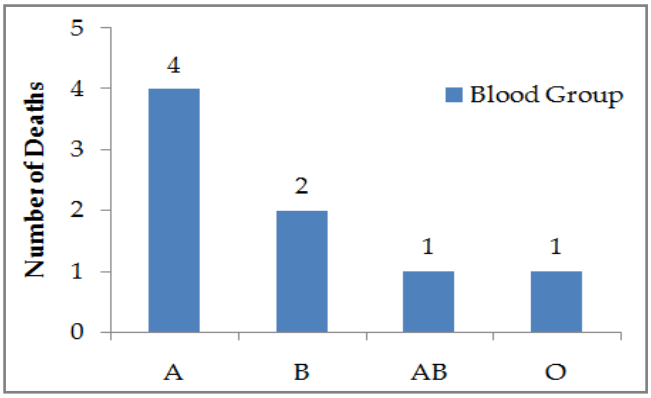

Figure: Association of mortality in COVID-19 patients with ABO blood groups.

DISCUSSION

Blood group antigens distribution is specific for different population groups and it has shown association with various infection and inherited disorders. Prevalence of these infections and inherited disorders give advantage of certain antigens over others. Previous studies had linked relationship of blood groups with a number of different diseases and infections. Infection of SARS-Co V-1 was related with type A-8, *H. pylori* with type O[9], Norwalk virus with type O[10], hepatitis B virus with type B followed by O[11], Malaria with type A and B[12], hypertension[13], type 2 diabetes mellitus[14] and many more.

The current project was designed to see the association of COVID-19 with ABO blood types. We compared the blood groups of general population with infected corona positive patients. This study will guide the clinicians to modify their treatments according to blood groups of patients. The blood groups presence in 2327 general population of Pakistan in latest studies showed a percentage distribution of A-527 (22.7%), B-945 (40.6%), AB-236 (10.1%) and O-619 (26.6%)[15,16,17]. Where as 326 COVID-19 infected patients in our study showed a blood group distribution of A-122 (37.4%), B-107 (32.8%), AB-26 (8.0%) and O-71 (21.8%). The current project was designed to see the association of COVID-19 with ABO blood types. This study will guide the clinicians to modify their treatments according to blood groups of patients.

In the following study we found a diverse association of ABO blood groups with SARS-CoV-2 infection causing COVID-19 disease. The A blood group substantial found to have increased risk of developing COVID-19 infection whereas O blood type was found to have a decreased risk of the disease. This explains that the ABO blood type is a distinction biomarker for the vulnerability of COVID-19 disease. The results are same with other two studies of corona virus infection SARS-CoV-1 by Cheng *et al* in 2005[8] and Chan *et al*, in 2003[18], with similar patterns of blood group types, in which O type were significantly less among SARS patients. Study done by Cheng *et al*[8], stated that A blood type has strong positive association for SARS-Co-V infection and negative association with O blood type.

We compared our data with the two international studies, one done in China by Zhao *et al*[4], and the other done in New York City by Micheal *et al*[5], and showed consistent results having a significant associations with A and O blood type with a significant *p*-value of 0.02 for A and <0.01 for blood type O. The percentages of blood group of A, B, AB and O of COVID-19 were 122 (37.4%), 107 (32.8%), 26 (8%) and 71 (21.8%) respectively. The blood group A showed stronger association while O had a negative relationship. The ABO blood group distribution in normal population of city of New York/CUIMC, Wuhan city and Shenzhen city all have high prevalence of blood group O followed by A, B and AB respectively, while in our normal population has high prevalence of blood group B followed by O, A and AB. Study done by Micheal *et al* in New York City compared COVID-19 patients in hospital with blood types distribution to the population of the city and found a strong association of blood group A with COVID-19 infection and mortality whereas blood group O found a decreasing rate of association[5]. Another study by Zhao *et al*, collected data from two hospitals of Wuhan, Wuhan Jinyintan, Renmin Hospital and Third people Hospital in city of Shenzhen comparing each hospital COVID-19 blood types with the general population of that city[4]. Blood group A showed a strong association for COVID-19 patients and a strong lower association of O blood type was seen. The results of COVID-19 study showed dissimilarity of increase number of A blood group followed by O, B and AB in the above two mentioned international studies, comparing it with our COVID-19 patients which showed increased distribution of positive cases in blood group A followed by B, O and AB.

We also found strong evidence of association between blood groups among critically ill patients undergoing treatment, on ventilators or those expired due to COVID-19 infection. Other

systems underlying the ABO blood types may also be present and increase the susceptibility for COVID-19 infection.

LIMITATION OF STUDY

This was single centered research and the results cannot be generalized however the results are consistent with other similar studies. Future studies are required to verify it at molecular level.

CONCLUSIONS

In this study we found a marked relationship between the ABO blood types and a biomarker in COVID-19 vulnerability. Blood type. A persons are at increased danger of the disease while with O blood type have a decrease danger for COVID-19 infection and severity. The current research findings shown to have numerous possible medical associations of COVID-19 and that of blood types may exist. The clinicians must be vigilant in treating the A blood type SARS-CoV-2 infected patients and need to take additional cautious investigations and aggressive management considering their high mortality.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by authors.

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