Relationship between blood group phenotypes (ABO, Rh and Kell) and nCOVID-19 susceptibility – A retrospective observational study.

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Research Article

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Abstract

Since the outbreak of coronavirus disease-19 research has been continued to explore multiple facets of the disease. The objective of the present study is to evaluate the relationship between blood group phenotypes and COVID-19 susceptibility.

In this hospital based, retrospective observational study 132 COVID-19 patients were enrolled from SMS Medical College and attached Hospitals, Jaipur, India after the proper approval from the institutional ethics committee. The ABO, Rh and Kell blood group phenotypes and demographic data of the patients were recorded. The observed proportions of ‘A’, ‘B’, ‘AB’, ‘O’, ‘Rh’, and ‘Kell’ blood groups phenotypes in COVID-19 patients were compared against the expected proportions (our null hypothesis) of the general population using multinomial tests and partition analysis.

There were significant differences between observed and expected frequency in ABO and Kell blood group phenotypes. Further partition analysis of ABO blood phenotypes showed that the group ‘A’ phenotypes were more susceptible for COVID-19. The Kell negatives were also more susceptible. The blood groups ‘AB’, ‘B’, ‘O’, and ‘Rh’ phenotypes showed no significantly different susceptibility for COVID-19.

The study shows relationship between ABO, Rh, and Kell blood group phenotypes and COVID-19 susceptibility. The application of these relationship in clinical practice requires more exploratory studies.

The following core competencies are addressed in this article: Screening Tool, Epidemiology, Patient Management, Medical Research.

Introduction

According to the World Health Organization report on 26 May 2020, there have been 5,370,375 confirmed cases of COVID–19, including 344,454 deaths globally [1]. The novel coronavirus 2019 (nCOVID–19) has spread beyond the boundaries of Wuhan (China) across the world. Researchers in many countries are now engaged studies of nCOVID–19. A number of diseases have been found associated with various blood group phenotypes. The ABO blood group antigens are widely distributed throughout the body along with the surface of red blood cells [2]. The association of ABO groups with malaria, pancreatic cancer, duodenal ulcer and other diseases has been elucidated [2–4]. Microorganisms interact with antibodies against blood group antigens, including ABO, T, and Kell systems [5]. The present study was undertaken to evaluate the relationships between the ABO, Rh, and Kell blood group systems and susceptibility for SARS-CoV–2 infection.

Patients And Methods
A hospital-based, retrospective observational study was designed to evaluate ABO, Rh, and Kell blood group phenotypes and susceptibility to SARS-CoV–2 infection. One hundred and thirty two laboratory confirmed, real-time RT-PCR (reverse transcription polymer chain reaction) positive cases of COVID–19 were enrolled in the study from SMS Medical College, Jaipur, Rajasthan, India. The mean age of the participants is 36.68 years (SD = 17.87 years) and men to women ratio is 8:3. The patients were treated according to the standard protocol of the institute. The ABO, Rh, and Kell blood group phenotypes and demographic data were collected for COVID–19 patients. The ABO, Rh (D antigen) and Kell phenotyping was carried out by a fully automated solid phase red cell adherence (SPRCA) technology [6]. The observed frequency of the blood groups—‘A’, ‘AB’, ‘B’ and ‘O’, ‘Rh positive’, ‘Rh negative’, ‘Kell positive’, and ‘Kell negative’ was calculated. The general proportions (expected proportions) of the above blood groups were obtained from a study of the North Indian population [7]. The expected proportions of ABO blood groups ‘A’, ‘AB’, ‘B’ and ‘O’ were 22.3%, 8.9%, 39.2% and 29.6%, respectively (Figure 1). Rh (D) phenotype was found positive in 93.8% and negative in 6.2%. For the Kell system, only 1.6% were Kell positive, and the remaining were Kell negative. The expected frequency of the blood group antigens was calculated by multiplying the total number of COVID–19 cases (n = 132) with the general proportions of the respective blood groups (Table 1).

**Statistical analysis**

The observed frequency of ABO blood groups, viz, ‘A’, ‘AB’, ‘B’, and ‘O’ were compared against the expected population frequency using multinomial test. The further hypotheses were tested using partition analysis [8]. Similarly, the observed and expected frequency of the Rh and Kell antigen system were compared using binomial test. The level of significance was considered at 5%. The database was maintained in MS Excel 2010 and JASP (version 0.12.2) software was used for the analysis [9].

**Results**

The observed frequencies of ABO, Rh and Kell blood group phenotypes with 95% confidence intervals were calculated (Figure 2). The comparison of the observed and expected frequencies of blood group phenotypes are shown in Figure 3.

**Comparison of phenotypes of ABO Blood group system for COVID–19 susceptibility**

The comparison of observed frequency and expected frequency of the ABO blood groups was performed using multinomial test (Table 1 and Figure 3).

There were significant differences between expected frequency and observed frequency in at least one of the blood group phenotypes ($\rho = 0.013$). The multinomial test does not reveal in which phenotype the difference actually exists. If the observed frequency is less than the expected frequency in one cell, it has
to be more in one or the other cells because the total for both the observed and the expected frequencies is the same. The observed frequency was higher in blood group ‘A’ and lower in blood groups ‘AB’, ‘B’, and ‘O’. To confirm the difference in observed frequencies across blood groups, a partition analysis was performed. ABO blood group phenotypes were further divided into two sub-groups and two hypotheses were tested using multinomial test (Table 2). First, the observed and expected frequency of blood groups ‘AB’, ‘B’, and ‘O’ were tested and no significant difference was found ($p = 0.89$). Second, the observed and expected frequency of blood group ‘A’ and ‘non-A’ was tested and found to differ significantly ($p = 0.001$). The partitioning of table 1 was done and the partitioned $x^2$ was calculated (Table 2).

It should be noted that the value of $x_T^2$ is very near to sum of partitioned $x^2$ values, that is

$$x_T^2 \approx x_{I}^2 + x_{II}^2 = 0.23 + 10.59 = 10.82$$

It can be inferred from the above analysis that persons with blood group ‘A’ are more susceptible to COVID–19 than ‘non-A’ groups. Further, there were no significant relationships between the ‘AB’, ‘B’, and ‘O’ blood group phenotypes and COVID–19 susceptibility.

**Comparison of Rh (D antigen) System for COVID–19 susceptibility**

The observed and expected frequency of Rh blood group phenotype showed no statistically significant differences ($p = 0.51$) (Table 3).

**Comparison of Kell antigen System for COVID–19 susceptibility**

The observed and expected frequency of Kell blood group phenotypes showed statistically significant differences ($p < 0.001$) (Table 4). The Kell negatives are more prone to COVID–19 infection as compared to Kell positives.

**Discussion**

The blood group antigens are an example of polymorphic traits inherited among individuals and populations. There are 34 recognized human blood groups and hundreds of individual blood group antigens and alleles. The variation in blood group system leads to variations in host susceptibility to many infections. Microorganisms interact with antibodies against blood group antigens, including ABO, T, and Kell systems [5]. The blood group antigens of the H, ABO, Lewis, and historical ‘P’ blood groups contains small carbohydrate epitopes expressed as post-translational modifications on glycoproteins, mucins, and glycolipids. The ABO system (International Society of Blood Transfusion [ISBT] 001) contains two structurally related carbohydrate antigens, A and B. The O or H antigen is the biosynthetic
precursor of A and B antigens and is listed under a separate blood group system (ISBT 018). All three antigens consist of 2 to 3 terminal oligosaccharides on glycoproteins and glycolipids [10].

It has been observed in the present study that people with blood group ‘A’ are more susceptible to COVID–19 than ‘non-A’ groups. Further there were no significant relationships between the ‘AB’, ‘B’ and ‘O’ blood groups and COVID–19 susceptibility. No relationship was found between Rh (D) antigen and SARS-CoV–2 transmission. Also, the Kell negative seems to be more susceptible to SARS-CoV–2 infection.

Goker (2020) investigated ABO blood group relationship on 186 patients and found blood group A (57%) was the most frequently detected blood group amongst the COVID–19 patients, followed by the blood group O (24.8%). Though no association between blood group and clinical outcome was established. The Blood group A individuals were significantly higher in number suffered with COVID–19 compared to controls (57% vs 38%, p <0.001; OR: 2.1). While the frequency of blood group O was significantly lower in the COVID–19 patients, compared to the control group (24.8% vs 37.2%, p: 0.001; OR: 1.8) [11]. Likewise, a retrospective cohort study with 265 patients from the Central Hospital of Wuhan showed there is a higher proportion of patients infected with SARS-CoV–2 that have blood group ‘A’ than that in healthy controls (39.3 % vs. 32.3 %, p = 0.017), while the proportion of blood group ‘O’ in patients infected with SARS-CoV–2 was significantly lower than that in healthy controls (25.7 % vs. 33.8 %, p < 0.01) [12].In other recent findings, Ziadi (2020) agreed the decreased efficiency of adhesion of Spike protein to ACE2 receptor by antibody A as suggest by many studies. He argued, that lower susceptibility of blood group ‘B’ and ‘O’ is true but it does not explain susceptibility of ‘AB’ blood group without anti A and anti B in serum [13].

In contrast, a study on 397 patients found an association of higher rate of COVID–19 infection with the ‘AB’ blood group [14]. However, the other findings of Abdollahi (2020) regarding no significant relationship between Rh phenotypes with COVID–19 susceptibility are consistent with our results [14]. One of the study analyze data from anti-A and anti-B antibodies viewpoint rather than ABO blood group and found subjects with anti-A (i.e. B and O blood groups) are significantly less susceptible to COVID–19 than those lacking anti-A whatever the group whereas there was no significant difference versus circulating anti-B in serum [15]. Similarly, Guillon et al. (2008) andGustafsson et al. (2005) argued that either a monoclonal anti-A antibody or natural plasma anti-A present in blood group ‘O’ specifically inhibited the SARS-CoV S protein/ACE2-dependent adhesion to ACE2-expressing cell lines. Therefore, ABO polymorphism could contribute to substantially reducing SARS-CoV transmission [16–17]. The results from Gustafsson et al., (2005) confirm that the glycans have more potential to carry variations than proteins and nucleic acids. The well-known example is polymorphic terminal glycosylation of the ABO blood group family of antigens. The association between infectious diseases and ABO antigens at mucosal surfaces leads to differential adherence of pathogens. The another way the blood group types might affect susceptibility is interaction with coagulation system. Some factors like von Willebrand factor and factor VIII affect in vivo half life and clearance of blood type antigens [18]. Similarly, Dai et al. (2020)studied the interaction among COVID–19 cases, hypertension, and ABO blood grouping. He found that the in hypertensive patients the renin-angiotensin-aldosterone system (RAS) is overexpressed due to
inhibitors of angiotensin converting enzyme (ACE2). The expression of ACE2 receptors is upregulated in hypertensives. The ACE2 receptor is the primary entry site for SARS-CoV–2. Dai (2020) mentioned that the ABO blood group is associated with ACE activity and ACE inhibitors induced cough. The GATC haplotype of the ABO gene polymorphism is prevalent among the non-O blood type patients and is positively associated with an ACE activity. Therefore, O blood type carriers have lower ACE levels and are less susceptible to COVID–19 infection [19].

There may be other mechanisms responsible for higher susceptibility of particular blood group phenotypes that require further studies.

**Conclusion**

The study shows relationship between ABO blood grouping and COVID–19 susceptibility, with group A being more susceptible. The application of these relationship in clinical practice requires more exploratory studies.

**Limitations Of The Study**

The expected proportions used in this study lacked information regarding age and sex, therefore, a multivariate analysis to adjust the effect of the two factors was not possible. However, this may not necessary as distributions of blood group phenotypes were similar across age and gender.

**Declarations**

As per the 'National Ethics Guidelines for Biomedical and Health Research' issued by Indian Medical Medical Research (ICMR) the consent of patients is waived (No 523 MC/EC/2020).

**Conflicts of interest**

There are no conflicts of interest between authors.

**Abbreviations**

ACE2: angiotensin converting enzyme inhibitors

ISBT: International society of blood transfusion

nCOVID-19: novel coronavirus disease-2019

p: p value

RT-PCR: reverse transcriptase polymer chain reaction
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

SD: standard deviation

SPRCA: solid phase red cell adherence

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Tables

Table 1. The observed frequencies of ABO blood group phenotypes in 132 laboratory confirmed COVID-19 patients and expected frequency in general population (Garg et al., 2015).

| Blood Group Phenotype | Total |
|-----------------------|-------|
| Observed frequency    |       |
| A                     | 45    |
| AB                    | 9     |
| B                     | 46    |
| O                     | 32    |
| 132                   |       |
| Expected frequency    |       |
| Observed frequency    |       |
| A                     | 29.43 |
| AB                    | 11.74 |
| B                     | 51.74 |
| O                     | 39.07 |
| 132                   |       |
| $c_T^2 = 10.79$       |       |
| degree of freedom = 3 |       |
| $P$ value = 0.013     |       |

*H$_0$ is the null hypothesis, which have the expected proportions of A : AB : B : O phenotypes are 0.22 : 0.09 : 0.39 : 0.30, respectively.
(Garg et al., 2015) (from Table 1) into two sections (Part I and II). Part I includes observed and expected frequency of AB and B blood groups, and part II include observed and expected frequency of ‘A’ and ‘O’ blood groups.

**Part I**

| Blood Group Phenotypes | Total |
|------------------------|-------|
|                        | AB    | B    | O    |
| Observed frequency     | 9     | 46   | 32   | 87   |
| Expected frequency under $H_{0A}^*$ | 9.96  | 43.89 | 33.14 | 87   |

$c_1^2 = 0.23$; degree of freedom = 2; $P$ value = 0.89

**Part II**

| Blood Group Phenotypes | Total |
|------------------------|-------|
|                        | A     | Non-A |
| Observed frequency     | 45    | 87    | 132  |
| Expected frequency under $H_{0B}^{**}$ | 29.43 | 102.57 | 132  |

$c_{II}^2 = 10.59$; degree of freedom = 1; $P$ value = 0.001

$H_{0A}$ is the null hypothesis with the expected proportions of AB : B: O phenotypes are 0.12 : 0.50 : 0.38, respectively.

$H_{0B}$ is the null hypothesis with the expected proportions of A : Non-A phenotypes are 0.22 : 0.78, respectively.

Table 3. The observed and expected frequencies of Rh antigen system in 132 laboratory confirmed COVID-19 patients.

| Rh D antigen System | Total |
|---------------------|-------|
| Rh Positive         | 122   | 10    | 132  |
| Rh Negative         |       |       |      |

$C_{Rh}^2 = 0.43$; degree of freedom = 1; $P$ value = 0.51

$H_{0Rh}$ is the null hypothesis with the expected proportions of Rh Positive : Rh Negative phenotypes are 0.94 : 0.06, respectively.

Table 4. The observed and expected frequencies of Kell antigen system in 132 laboratory confirmed COVID-19 patients.
|                   | Kell antigen System |          |          |
|-------------------|---------------------|----------|----------|
|                   | Kell Positive       | Kell Negative | Total   |
| Observed frequency| 123                 | 9        | 132      |
| Expected frequency under $H_0^K$* | 129.89              | 2.11     | 132      |

$c_K^2 = 23.83$; degree of freedom = 1; $P$ value < 0.001

$H_0^K$ is the null hypothesis with the expected proportions of Kell Positive : Kell Negative phenotypes are 0.98 : 0.02, respectively.

**Figures**

![Figure 1](image)

**Figure 1**

Frequency of ABO blood phenotypes (a) Observed frequency in COVID-19 (b) Expected frequency in general North Indian population (Garg et al., 2015)
Figure 2

Observed proportions of A, AB, B, O, Rh positive, Kell positive blood group phenotypes with 95% confidence intervals based on independent binomial distributions of 132 laboratory confirmed covid-19 patients.
Figure 3

Comparison of observed and expected proportion of blood group phenotypes from a random sample of 132 laboratory confirmed covid-19 patients and study on North Indian Population (Garg et al., 2015), respectively.