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Original Research article

Descriptive study on the distribution of ABO blood group and Rh type in the mechanically ventilated patients with COVID 19 disease

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Abstract

Introduction: COVID-19 Pandemic was supposedly one of the deadliest events in the 21st century which has caused immense strain on the healthcare and human resource. Globally this has caused 450 million of infective cases and over 6 million deaths till now. Studies have shown that ABO polymorphism plays a vital role in occurrence of various communicable and non-communicable diseases. ABO polymorphism was also considered to play a significant role in susceptibility and progression of SARS-CoV-2 infection by various studies. Though the results are not equivocal, globally researchers are interested in identifying the trend of infection and severity of illness among particular blood groups which could be helpful in framing certain prognostic markers for the COVID 19 disease and also can aid the researchers in inventing the vaccines and the novel therapies in curing the COVID 19 disease. This can also lay the foundation for some genomic studies which can link certain blood groups with certain novel diseases in the era of biological warfare.
Aim: To find the distribution of ABO and Rh Typing of blood groups among mechanically ventilated patients in COVID 19 disease.
Materials and Methods: A retrospective study was conducted in Government Designated Dedicated COVID 19 Hospital of Chamarajanagar district of Karnataka state, India over a period of 10 to 11 months wherein retrospective data was collected from June 2020 to March 2021. It was a time bound study and no sample size was calculated. Study group included 730 Confirmed RT PCR/Rapid Antigen Test positive for COVID-19 patients admitted in intensive care unit with severe COVID illness who required oxygen supplementation and control group included 3217 regular blood donors of blood bank, Chamarajanagar institute of medical sciences, Chamarajanagar. Informed consent was waived because of absence of any patient identifying information and the urgent nature of the investigation and ethical committee clearance was obtained from the institute. The difference in proportion of distribution of blood groups between study and control groups was compared using Z test. AP value < 0.05 was considered statistically significant.
Results: Majority of the patients who had severe COVID-19 manifestations belonged to the age of more than 40 years contributing to 94.8% and males formed the majority (67.0%). It

was also shown that proportion of patients with blood group A positive was 33.4% in ICU group of patients who were mechanically ventilated compared to 24.6% in the control group which represented general population with P value of <0.005 which was statistically significant which suggests that the severity of illness was more in patients with A positive blood group. Where as other blood groups didn't show this kind of association.
Conclusion: This study provides evidence that the patients of severe COVID 19 disease with a positive blood group were more likely to require mechanical ventilation compared to other blood groups.

Keywords: SARS-CoV-2; disease severity, mechanical ventilation

Introduction

In December 2019 world had seen the first case of COVID-19 which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of 15th March 2022, globally over 450 million of infective cases and 6 million deaths have been reported [1]. SARS-COV-2 is a β-corona virus that is highly homologous to SARS-CoV-1 and uses Angiotensin-Converting Enzyme 2 (ACE2) as a cellular receptor. Many cells of human body express ACE2 receptors conferring the capacity to infection by SARS CoV2 [2].
The most common symptoms of COVID-19 involved fever, dry cough and fatigue followed by myalgia, anorexia, dyspnea, and can even be acute respiratory distress syndrome or bilateral pneumonia [3, 4].
After the outbreak of the COVID-19 infection, the association between blood group polymorphisms and disease severity was studied. One of the early studies which were conducted with more than 2000 patients showed that the incidence of occurrence of COVID 19 and the severity was more frequent with group A phenotype than group O phenotype [5, 6, 7, 8]. Authors of this study did a multicentre cohort study enrolling critically ill COVID 19 patients admitted in various Intensive Care Units of 67 Hospitals in United States of America. They studied the association between clinical outcome and ABO Phenotype of these patients. They concluded that white patients with blood group A were at higher risk of critical illness due to COVID 19 whereas type O blood group patients were protected [6].
Another study conducted by Abdollahi A *et al*. concluded that group AB patients were at more risk of getting infected whereas group O patients were protected [7]. However Latz CA *et al*. concluded that the susceptible group was Group B and Group AB and Group O being protected by the infection [9]. Where as in a study done on Indian Healthcare workers by Mahajan *et al*. it was found that blood group A and AB healthcare workers were more susceptible for the COVID 19 infection [10].
These varying results are probably because studies did not account for confounders like age and blood group distribution among general population. So the present study was planned with the aim of considering demographic data like age, sex distribution among critically ill COVID 19 patients and to analyse the distribution of blood group among those patients in comparison with blood group distribution among general population.

Materials and Methods

This was a retrospective study which was conducted at a tertiary care centre and a teaching hospital, Chamarajanagar Institute of Medical Sciences and hospital, Chamarajanagar which was a designated Dedicated COVID Hospital (DCH).
Literature review, synopsis preparation and IEC clearance was done during October 2020 to November 2020 and data related to study patients which were admitted in Intensive care Unit from June 2020 to March 2021 was collected retrospectively. It was a time bound study and no sample size was calculated.

The study included two groups, the study and the control group. Adopting a purposive sampling the study group included all the severely ill COVID 19 patients who were treated in Intensive Care Unit from June 2020 to March 2021with documented blood group type. Out of 920 patients admitted to our ICU during the study period only 730 patients met the inclusion criteria, and considered as study group. The general population who actually were the voluntary donors of blood at Chamarajanagar Institute of Medical Sciences and Hospital Blood Bank which included 3217 subjects were considered as the control group.

Study group
Inclusion criteria

- Confirmed RT PCR/Rapid Antigen Test positive for COVID-19.
- Patients of all age group with various co morbidities on various medications.
- Admitted in intensive care unit requiring oxygen supplementation which included moderate to severe COVID 19 pneumonia, severe ARDS, sepsis and septic shock.
- Available records of the patients with documented demographic data, blood group type and disease progression data.

Exclusion criteria

- Negative for RT PCR/Rapid Antigen Test for COVID-19.
- Confirmed RT PCR/Rapid Antigen Test positive for COVID-19 but who never required oxygen supplementation.
- Readmission of the patient who again contracted COVID 19 illness with moderate to severe illness.
- Unavailability of records of demographic data, blood group type and disease progression data.

Control group
Inclusion criteria

- The voluntary donors of blood at Chamarajanagar Institute of Medical Sciences and Hospital Blood Bank, who had donated blood during the same period.

Exclusion criteria

- Repeated blood donors were excluded from the list as it would confound the result.

Immunisation status was not available as it was not started during the study period. All the patients were given standard protocol based treatment uniformly. The study manuscript got an approval by the Ethical Committee of Institute of Medical Sciences, Chamarajanagar with an IEC Ref No: CIMS/IEC-02/43/2020. As a part of routine medical procedure all the data was collected. Informed consent was waived by the Ethical Committee as the study was retrospective with subset of prospective in nature, absence of any patient identifying information and the urgent nature of the investigation.

Statistical analysis

Data was entered in excel and analysed using SPSS version 20.0. Results were presented as proportions. The comparison of blood group distribution between two groups was done by Z test. A P value < 0.05 was considered statistically significant.

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Results

Majority of the patients who had severe COVID-19 manifestations belonged to the age of more than 40 years contributing to 94.8% and males formed the majority (67.0%). [Table/Fig 1]

Table1: Distribution of the study group individuals based on age and gender

Age-group	Males (N=489) N (%)	Females (N=241) N (%)	Total (N=730) N (%)
≤20	01 (0.2)	01 (0.4)	02 (0.3)
21-40	24 (4.9)	12 (5.0)	36 (4.9)
41-60	214 (43.8)	131 (54.4)	345 (47.3)
>60	250 (51.1)	97 (40.2)	347 (47.5)

On comparing the distribution of the blood groups among the severe COVID-19 subjects who required mechanical ventilation, it was found that the proportion of patients with blood group A +ve was 33.4% in study group compared to 24.6% in the control group which represented general population which was statistically significant with P value of <0.05, which suggests that the severity of illness was more in patients with A+ve blood group. [Table/Fig 2]

Table2: Comparison of the distribution of the blood groups among the study andcontrol groups

Blood-group	Study group (N=730) N (%)	Control group (n=3217)N (%)	z-value (*P*-value)
A +ve	231 (31.6)	790 (24.6)	3.95(<0.005)
A –ve	03 (0.4)	33 (1.0)	-1.58(0.11)
B +ve	176 (24.1)	888 (27.6)	-1.92(0.05)
B –ve	02 (0.3)	23 (0.7)	-1.36(0.17)
O +ve	255 (34.9)	1226 (38.1)	-1.60(0.11)
O –ve	12 (1.7)	48 (1.5)	0.30(0.76)
AB +ve	46 (6.3)	184 (5.7)	0.61(0.54)
AB –ve	05 (0.7)	25 (0.8)	-0.26(0.79)

* indicates statistical significance at *p*(<0.05).
Note: Z test for difference in proportions was used as a test of significance in the above table.

In the same context, although the proportion of O +ve patients dominated the table in both the study group (34.5%) and control group (38.1%), the difference was not significant with P value of 0.11. This was similar to other blood groups where significant difference was not found among study and control groups. [Table/Fig 2, Table/Fig 3]

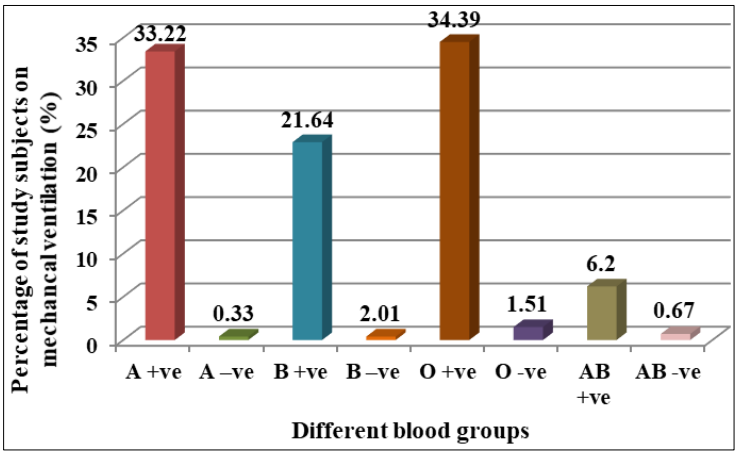

Fig 1: Percentage distribution of severe COVID-19 subjects with different blood groups on mechanical ventilation (Invasive and Non-invasive) (n=730)

Table 3: Percentage distribution of severe COVID-19 subjects with different blood groups on two types of mechanical ventilation (Invasive and Non-invasive) (N=730)

Blood-group	Study group (N=730) N (%)		
	Noninvasive Ventilation	Invasive ventilation	Total ventilation
A +ve	180 (24.65)	51 (6.9)	231 (31.6)
A –ve	3 (0.4)	0	03 (0.4)
B +ve	144 (20)	32 (4.1)	176 (24.1)
B –ve	02 (0.3)	0	02 (0.3)
O +ve	215 (29.4)	40 (5.5)	255 (34.9)
O –ve	10 (1.39)	2 (0.29)	12 (1.7)
AB +ve	37 (5)	9 (1.3)	46 (6.3)
AB –ve	4 (0.56)	1 (0.16)	05 (0.7)

Of total 730 COVID-19 patients who were mechanically ventilated, 126(17.26%) patients were ventilated by invasive mode of ventilation.

Discussion

Since the time Landsteiner discovered the presence of ABO group and Rh group antigens on the erythrocyte membrane, over a period of time it was also known that these antigens are expressed in columnar epithelial cells of respiratory tract and also on endothelial cells of the blood vessels.

Glycosyltransferases are encoded by allelic genes located on chromosome 9. Antigen A carries N-acetylgalactosamine, whereas B antigens carries d-galactose added to the glycoprotein H antigen by the respective Glycosyltransferases. The O antigen carries unmodified H antigen due to the absence of these transferases following a deletion on the allelic gene. 'Naturally occurring antibodies' like anti A and anti B are produced as a result of exposure with non-self A and/or B antigens, which are often found in the gut microbiota early in life [11]. Antibodies are predominantly IgG type in individuals with O blood group whereas IgM isotype is naturally found in those who lack the respective antigens [5, 12-14].

Individuals with certain blood groups are more prone for certain communicable diseases and this has been well documented in the past, noted once are malaria and cholera [15-18].

In one of the study it was observed thatin the COVID 19 group, subjects lacking anti A antibody in serum such as blood group A and Group AB were statistically more represented than group B and Group O who were having anti-A antibody in serum, whereas there was no significant difference with respect to circulating anti-B[5]. In addition it was also observed that Group O was significantly less represented in comparison with Group B even though both of them were lacking the supposedly protective anti A antibody which shows that anti A antibody of Group O confreres more protection than Group B. Probably because of the predominance of IgM isotype in group B individuals compared to IgG isotype in group O individuals [14].

The true causal association between ABO blood group and COVID-19 is influenced by many factors like the study population, age, sex, associated medical disorders and severity of COVID illness and also on the selection of the appropriate control group [19, 20]. It was estimated that geriatric population especially more than 60 years and male gender are prone to complications. More than 75% of COVID deaths is seen in patients with coexisting medical disorders [21].

The probable mechanisms have been hypothesised; ABH antigens can act as receptors for pathogens and its virulence factors and toxins. The glycosylation of the receptors on the mucosal surfaces have been affected by the Glycosyltransferases of group A and B subjects which can influence the viral entry and inoculation at various steps. It also inhibits the pathogens by acting as natural antibodies and lectins. Studies have shown that naturally

occurring IgG anti-A isoagglutinins in group O individuals inhibit the attachment of virus to ACE2 receptor [2]. It has also been hypothesised that when A or B individuals are infected with the COVID virus, S protein of the virions will be loaded with the respective antigens on its surface. When this virus infects an individual with blood group O who has natural isoagglutinins like anti A anti B, it will prevent the infection by inhibiting its interaction with ACE2 receptor. Hence blood Group O individuals with circulating natural antibodies would benefit the most against COVID 19 severe illness. Whereas individuals with Group A and AB can easily get infected with SARS-CoV-2 viruses transmitted from group A individuals which are expressing A antigens and hence they cannot infect easily those individuals with Group B or O who has natural anti A antibodies circulating in them. Similarly, individuals with Group B and AB can easily get infected with SARS-CoV-2 viruses transmitted from group B individuals.

In patients with COVID-19 illness there might be an antigen antibody reaction on the viral envelope by A and B antigens on the surface with anti A and anti B antibodies in the plasma which might prevent the infection of the target cells. The entry of virus into lung epithelium could also be prevented by anti A antibodies bound to S protein on the SARS virus which may block its interaction with ACE2 receptors. Increased cardiovascular complications in group A individuals infected with SARS CoV is probably because of increase in ACE 1 activity found in Group A individuals [22]. Similarly increased thromboembolic complications in group A individuals is probably because of increased VWF and Factor VIII levels in them [23]. Possibly the S proteins of COVID virions which are carrying ABH antigens might influence the attachment of virus with ACE 2 receptor, or even these glycans if present on target cells could act as alternative low affinity receptor for the virus or its virulence factors.

When we considered the influence of demographic characteristics on the severity of COVID illness it was found that 94.8% of the study population belonged to age group more than 40 years and male gender (67%) had higher risk than female gender (33%). It was similar to a conclusion drawn by a meta-analysis which comprised 20 studies with more than 64000 COVID patients which showed patients with age group above 50 years were nearly 15 times more likely to have adverse outcome in comparison with patients below 50 year old ($P < 0.00001$). Similarly male patients were nearly 2 times more likely to have adverse outcome in COVID 19 illness compared to female patients ($p < 0.00001$) [24].

It was observed invarious studies that in many of the patients ranging from mild to severe pneumonia proportion of group A individuals was higher [6, 8] and in some studies the susceptible group was group B and group AB [7, 9] whereas lower risk was present in Group O individuals compared to healthy controls. The authors of one of the largest Meta regression analyses even proposed that nations with higher group O prevalence had lower mortality [25]. Considering the influence of Rh typing, SARS COV 2 infection was more common in Rh(D) positive patients [9].

In the current study, although the proportion of O +ve patients dominated the table in both the study group (34.5%) and control group (38.1%), the difference was not significant with P value of 0.11 which shows that group O being the most prevalent blood group in the region was also predominantly represented in the study group but the difference was not statistically significant. However, this study also shows that individuals with blood group A are more likely to have severe COVID 19 illness requiring mechanical ventilation as depicted in the results wherein group A individuals represented 31.6% in study group compared to 24.6% in control group which represented the general distribution of blood group. The difference was statistically significant with a p value of < 0.05. Results of this study are in coherence with another multi-center retrospective cum prospective study of severely ill COVID 19 intensive care patients wherein data related to severity of illness, mechanical ventilation, laboratory parameters and Continuous Renal Replacement Therapy were analysed. This study shows that group O individuals represented 35% of the total mechanically ventilated patients

whereas group A individuals were 32% which is similar to the results obtained in the present study. They also concluded that intensive care unit stay (P=0.03) and mechanical ventilation requirement (P=0.02) was more in blood group A or AB compared to Group O or B. Current study also refuted the susceptibility variation among individuals based on Rh typing [26].
The clinical implication of this study is that it not only facilitates in understanding the possible role of the ABO antigens in the pathogenesis of COVID 19 illness but also will guide researchers in finding possible cure of the disease. Possibly we can predict the outcome of illness in COVID-19 patients by formulating a prognostic score including variables which are closely associated with bad outcome like age, sex and ABO and Rh grouping with other confounders like high BMI, cardiovascular disease, gender and diabetes mellitus etc.

Limitations

The limitations of this study is that study population included only RT PCR/ RAT positive patients which were the predominant category of patients during the first wave of COVID 19and excluded RT PCR/RAT negative COVID like syndrome patients which contributed significantly to the COVID patients in the second wave and later dates. The proposed control group was hypothesised to be taken as representative of whole district but there can be deviations from the true distribution and also demography of the control group could not be matched. Also this study did not account for the influence of underlying co morbidities which would lower the strength of immune system and hence could influence the outcome of the illness.
Further large studies are required to analyse the association between genetic polymorphism and the severe COVID 19 illness. In the era of biological warfare and bioterrorism wherein genetically engineered new infectious agents can be created, deciphering the relation between ABO blood group and severity of these kinds of infectious agents could aid the researchers in inventing the vaccines or therapeutic agents in handling such pandemics.

Conclusion

This study provides evidence that the among the critically ill patients admitted to Intensive Care Unit, the proportion of patients with A positive blood group were more likely to require mechanical ventilation compared to other blood groups. Since the blood group O was the predominant group in general population it also dominated the study table but statistically there was no significant difference. Hence this study shows that the blood group A has more negative impact on the progression of COVID 19 illness than other blood groups.

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