Association between ABO blood groups, rhesus and SARS-CoV-2 infection or severe COVID-19 disease in a population of western Algeria

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Abstract

Introduction: Since the onset of the SARS-CoV-2 pandemic in March 2019, scientists have been trying first to define the virus, the disease and even the factors that exacerbate the situation. Questions have arisen about the relationship between the patient’s blood type, rhesus and the development of a COVID-19 infection. The purpose of this study is to determine whether or not there is a correlation between blood types, rhesus and COVID-19 disease, as well as its severity.

Material and methods: We conducted a retrospective study of 7 months from August 2021 to February 2022, involving 59 patients with 33 male and 26 female subjects suspected of being infected with COVID-19, between the ages of 14 and 90. These patients were recruited from the Pasteur Institute “Oran Annex” and the Local Public Health Facility (EPSP BENI SAF). RT-qPCR techniques and blood grouping were used to determine the blood type of patients and whether or not they were infected with the SARS-CoV-2 virus. Patient information was retrieved from both facilities from medical records.

Results: According to our results, 83% of patients tested positive for SARS-CoV-2 and 17% tested negative. The proportion of the O-group among those infected with SARS-CoV-2 (66%) was lower than in healthy patients (70%), while the proportion of the A-group (20%) was higher in negative patients than in positive patients (17%). The percentage of blood type B was identical in the affected and healthy groups (10%), while the AB group was present in affected patients (7%) and was completely absent in healthy patients. The p-value was 1 and this indicates that the link between blood type and COVID-19 infection is not significant.

Conclusions: In light of our findings, there is a correlation between rhesus and the age of patients during the development of COVID-19 infection.

Key words: COVID-19, SARS-CoV-2, blood groups, rhesus, comorbidities.
Introduction

The Coronavirus disease 2019 (COVID-19) pandemic, since its discovery in 2019, has caused immense disaster worldwide, affecting more than 500 million people and more than six million deaths. COVID-19 occurs with a wide range of diseases ranging from self-limiting respiratory tract disease to multiple organ failure and ultimately death, especially in people with comorbidities.

A common pathophysiological dynamic is the use of host angiotensin converting enzyme 2 (ACE-2) to initiate direct viral invasion and access target cells [1, 2].

The first observation was made in China that most of the severe COVID-19 cases were Rhesus-positive individuals. There, doubts were beginning to build about the possibility that one blood group is affected more than the others. Two hypotheses were presented to justify the association between the blood type, rhesus and COVID-19 disease.

The first hypothesis is that some substances found free in blood plasma neutralize and weaken the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus responsible for COVID-19 disease and its virions.

The other hypothesis states that some blood groups tend to produce blood clots frequently seen in COVID-19 patients more than other groups [3].

The objectives of this retrospective and prospective study are to investigate a possible correlation between the ABO system and the development of COVID-19 infection and to demonstrate whether this is related to the development of severe forms of infection.

Material and methods

Study population

Our retrospective study was conducted between August 2021 and February 2022 (duration of 7 months) in patients suspected of being infected with SARS-CoV-2 from western Algeria (Oran, Ain Temouchent, Relizane, Tiaret). Our population consisted of 152 individuals while our final sample consisted of 59 suspect individuals who met the criteria of our study.

The study parameters were age, region, sex, ABO group, rhesus monkeys and COVID-19 results.

RT-PCR technique

To complete our current study, a molecular biology technique is highlighted/used real time polymerase chain reaction (RT-PCR). The method includes extracting SARS-CoV-2 virus from nasopharyngeal samples.

RT-PCR is the most reliable, unavoidable and most widely used method for the detection of SARS-CoV-2.

It consists of extracting the viral RNA by 2 different kits: NuActor and Biocomma. The Biocomma kit is non-toxic, painless with a plate that can extract up to 32 samples in 28 min.

While the NuActor kit contains a 20-ml bottle of lysis solution and a 48-well plate that extracts 8 nasopharyngeal samples in 12 min.

Subsequently, the PCR mix is prepared in a specialized room. In a tube, 1 µl of Taq Polymerase, 5 µl of the Master mix (containing dNTPs and reverse transcriptase) and 4 µl of extra pure water are added. After vortexing the tube, 10 µl of the mix are added to each well of the PCR plate. 10 µl of samples are added to each well with the first well for the negative control and the second well for the positive control and the plate is centrifuged at 1000 revolutions per minute.

Finally, the plate is deposited in the PCR apparatus for a period of 62 to 64 min.

Blood grouping technique

For the determination of the blood type, the technique of Beth-Vincent is highlighted. This test is universal and most widely used, thus facilitating blood transfusion and avoiding incompatibility of blood groups.

Beth-Vincent method identifies surface antigens found on blood erythrocytes using anti-A, anti-B, anti-AB and anti-D antibody solutions.

Statistical analysis

Statistical data analysis was performed using IBM SPSS version 20 software.

Results

Univariate analysis of the distribution of COVID-19 results by the ABO group

The distribution of ABO groups in our population is very varied. Group O patients are the majority in the case of a positive COVID-19 result than in the cases with a negative COVID-19 result (82% vs. 18%). For group A patients, the percentage is much higher in patients with a positive COVID-19 result than in negative patients (83% vs. 17%).

Group AB patients are completely absent in the case of a negative COVID-19 result but present in the case of a positive COVID-19 result (100%) (Figure 1 A).

Univariate Rhesus analysis

Patients with a positive COVID-19 result and a positive rhesus factor are the majority compared to patients with a negative rhesus factor (96% vs. 4.1%). Also, in patients with a COVID-19 negative result with a positive rhesus factor, the major-
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Univariate analysis based on comorbidity variable

In patients with COVID-19 positive results, the vast majority (69%) had no comorbidity. Thereafter, we observe type 1 diabetes (DT1) and hypertension (HTA) with a similar percentage (8.2%). Atrial fibrillation (ACFA) with hypertension and type 1 diabetes with hypertension are of the order of 4.1% each, while type 2 diabetes (DT2), bronchitis and DT2 with hypertension are of the order of 2% each. All patients with COVID-19 negative results reported no comorbidities (Figure 2).

Univariate analysis by age variable

The median for COVID-19 positive cases is 50.0, while the median for COVID-19 negative patients is 29.0 (Figure 3).

Univariate analysis according to sex variable

In our recent study, male patients were superior to female patients with a positive COVID-19 result (57% vs. 43%). However, patients with a COVID-19 negative result have a 50% gender equality (Figure 4).

Discussion

In our patient series, which included 59 patients with all of our study parameters, the number of patients affected (49 individuals) was higher than the number of healthy patients (10 individuals). Several negative patients were excluded because they did not meet our study criteria (60 patients tested negative). This figure is far from alarming when compared to the number of patients recruit-
ed from our Chinese collaborators: 105 positive and 103 negative patients, 1775 affected and 1919 healthy respectively [4, 5].

As noted in Table I, there is a different gender distribution in patients with 59.18% of males and 40.82% of females. This is consistent with the percentage of a Spanish study [6].

The p-value calculation by Fisher’s test gave a value of 0.74, but no significant difference and no association between sex and COVID-19 infection was noted. These results are consistent with our Chinese collaborators whose p-value = 0.15 [7].

The proportion of Group O in SARS-CoV-2 infected individuals was decreased compared to healthy individuals (66% vs. 70%), so the proportion of Group A was high in COVID-19 negative patients compared to COVID-19 positive patients (20% vs. 17%).

The percentage of blood group B was identical in the affected and healthy groups (10%) while

### Table I. Distribution of patients according to COVID-19 results by gender, rhesus and comorbidities

<table>
<thead>
<tr>
<th>Parameter</th>
<th>COVID+ results (n = 49)</th>
<th>COVID– results (n = 10)</th>
<th>N</th>
<th>P-value</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender, n:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28 (57%)</td>
<td>5 (50%)</td>
<td>33</td>
<td>0.74</td>
<td>Fisher</td>
</tr>
<tr>
<td>Female</td>
<td>21 (43%)</td>
<td>5 (50%)</td>
<td>26</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Rhesus, n:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>47 (96%)</td>
<td>7 (70%)</td>
<td>54</td>
<td>0.03</td>
<td>Fisher</td>
</tr>
<tr>
<td>–</td>
<td>2 (4.1%)</td>
<td>3 (30%)</td>
<td>5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Comorbidity, n:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>34 (69%)</td>
<td>10 (100%)</td>
<td>44</td>
<td>0.86</td>
<td>Fisher</td>
</tr>
<tr>
<td>DT1</td>
<td>4 (8.2%)</td>
<td>0 (%)</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HTA</td>
<td>4 (8.2%)</td>
<td>0 (0%)</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ACFA + HTA</td>
<td>2 (4.1%)</td>
<td>0 (0%)</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DT1 + HTA</td>
<td>2 (4.1%)</td>
<td>0 (0%)</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DT2</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DT2 + HTA</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

### Table II. Distribution of patients with COVID-19 results according to blood type

<table>
<thead>
<tr>
<th>COVID results (n = 49)</th>
<th>ABO group O (n = 39)</th>
<th>ABO group A (n = 10)</th>
<th>ABO group B (n = 6)</th>
<th>ABO group AB (n = 4)</th>
<th>N</th>
<th>P-value</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>32 (82%)</td>
<td>8 (80%)</td>
<td>5 (83%)</td>
<td>4 (100%)</td>
<td>49</td>
<td>1</td>
<td>Fisher</td>
</tr>
<tr>
<td>–</td>
<td>7 (18%)</td>
<td>2 (20%)</td>
<td>1 (17%)</td>
<td>0 (0%)</td>
<td>10</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

### Table III. Distribution of patients with COVID-19 results according to age

<table>
<thead>
<tr>
<th>COVID results</th>
<th>Mean (standard deviation)</th>
<th>Median (Q25–75)</th>
<th>Min.</th>
<th>Max.</th>
<th>N</th>
<th>P-value</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>50.0 (18.3)</td>
<td>50.0 (38.0–63.0)</td>
<td>14.0</td>
<td>90.0</td>
<td>49</td>
<td>&lt; 0.01</td>
<td>Mann-Whitney</td>
</tr>
<tr>
<td>–</td>
<td>31.9 (13.0)</td>
<td>29.0 (22.0–40.8)</td>
<td>14.0</td>
<td>53.0</td>
<td>10</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
group AB was present in the affected patients (7%) and was completely absent in the healthy patients. Our results are inconsistent with literature studies [4].

The statistical study by the Fisher’s test revealed $p = 1$ which shows no significance, i.e. the blood group has no association with COVID-19 infection (Table II). This is consistent with the Tunisian and Iranian studies with $p-value = 0.64$, $p-value = 0.392$ respectively [8, 9].

According to the data in Table I, the positive rhesus factor was present in the majority of patients with COVID-19 compared to the negative rhesus factor (96% vs. 4.1%), while the percentage of the negative rhesus factor was high in healthy patients compared to patients with COVID-19 (30% vs. 4.1%).

In addition, the statistical study by the Fisher’s test showed a $p = 0.03^*$, with a significant difference and indicating the presence of an association between rhesus and COVID-19 infection.

Our results are consistent with our Canadian and U.S. collaborators (RRA = 1, RDA = 1.22, respectively) [10, 11].

Our sample shows different age groups with a minimum of 14 years and a maximum of 90 years. In the COVID-19 positive group, the mean age was 49.98. Whereas in the COVID-19 negative group, it was 31.90. The statistical study, by the Mann-Whitney test in Table III, revealed a $p-value < 0.01^{**}$ which is highly significant.

Therefore, there is a strong association between age and COVID-19 infection, and this is consistent with our collaborators whose $p-value$ was $< 0.0001$ [7].

The majority of COVID-19 patients did not have comorbidities (69%). Table I showed, by Fisher’s Test, a $p-value = 0.86$, which is not significant, i.e. the comorbidity is not associated with the development of a COVID-19 infection. Our results are inconsistent with our employees with a $p-value$ of 0.001 [7].

In conclusion, this study was carried out on a population in western Algeria (Oran, Ain-Temouchent, Relizane and Tiaret). The patients concerned were suspected of being infected with COVID-19 during a period from August 2021 to February 2022 at the level of the microbiology and molecular biology laboratory of the Institut Pasteur d’Algérie ‘Antenne d’Oran’ and the EPH BENI SAF (Public Hospital of Beni Saf). Based on our results, we concluded that there is no correlation between blood groups, COVID-19 infection and its severity. O-positive patients have the same risk of developing virus infection. Rhesus and patient age may be risk factors for COVID-19 infection.

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The study was carried out at the Laboratory of Molecular Biology, Pasteur Institute of Algeria (IPA) Antenna of Oran and at the Department of Pneumo-Phthisiology and Infectious Diseases of the Local Public Health Establishment Béni Saf (EPSP Béni Saf).

**Conflict of interest**

The authors declare no conflict of interest.

**References**