Association between Blood Type and Severity of Covid-19 Infection among Patients in Kaski District, Nepal

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Abstract
Novel Coronavirus (SARS-CoV-2) first broke out in China and speeded globally. With the considerable number of Covid-19 infections and deaths, the situation has brought up major public health and governance concerns. This research study is conducted to understand if there is any association between blood group and Covid-19 disease severity. Study is conducted in Life care diagnostic and research center, New road Pokhara. A total of 1050 people who underwent RT-PCR test at Life Care Diagnostic and identified positive are used as the sample to conduct this research study. Our findings reveal that Covid-19 severity is uncommon in people with blood group O and Rh negative whereas those with blood group AB and B types are found to be severely affected. We also have an impression that individuals with rhesus negative (Rh-negative) blood groups have higher resistance for Covid-19 infection and severity.

Keywords
SARS-Co-2, SARS-CoV-2/Covid-19, Covid-19/Rh, Rh, Symptoms Severity, Kaski District, Nepal

1. Introduction
Covid-19 also called severe acute respiratory syndrome (SARS) is caused by SARS-CoV-2, a novel virus that belongs to the family Coronaviridae. Novel Coronavirus (SARS-CoV-2) was first reported in December 2019 in the Wuhan city, China [1]. On 31st December, 2019, cases of pneumonia, an unknown etiol-
ogy was detected in Wuhan city, Hubei Province of China. As informed by the WHO China Country office [2]. Later, on 11th March 2020, the outbreak was declared a global pandemic as it remained the global health crisis of our time, and the greatest challenge we have faced since World War II [3]. From its emergence in Asia in 2019, the virus has spread to every continent.

In Nepal, the first Covid-19 case was confirmed on 23rd January, 2020 in a 31-year-old Nepali student who had returned from China. Similarly, the first death due to Covid-19 in Nepal occurred on 14th May, 2020 [4]. By the end of March 2021, Nepal had 277,461 Corona positive cases as registered and 3031 deaths occurred [5].

With increased number of illnesses and deaths associated with Covid-19, scientists are eager in presenting the data that characterized individuals susceptible to Covid-19 infection, and the risk factor that may be associated with disease progression and severity [6]. Many scholars and scientists from all over the world have been working relentlessly in finding the association between blood groups and SARS-CoV-2.

Article “Susceptibility to the novel coronavirus disease (Covid-19) is associated with ABO and Rh blood groups: a case-control study from Afghanistan” by Saify et al. took a sample of 301 Covid-19 confirmed cases in their study. The findings show that the blood group with RH− compared to the RH+ phenotype strongly increased the risk of Covid-19 (OR = 2.97, 95% CI 1.86 - 3.89, p < 0.001). The study further reveals that O− vs O+ phenotype is significantly associated with the risk of Covid-19, which is quite similar to the association between Rh− and susceptibility to Covid-19 [7].

Likewise, “Association between blood type and Covid-19 infection, intubation, and death” by zietz et al. shows that type A blood was at decreased risk of intubation, and death compared to type O, while type AB was at increased risk of both outcomes. Additionally, type B individuals were at higher risk of intubation but at lower risk of death, compared with type O. Those with negative Rh (D) were at decreased risk for both intubation and death, consistent with a lower risk of initial infection [8].

In contrast to the above two findings, the study from MGH news and public affairs found that there is no relationship between Covid-19 and blood type. This study was done by Harvard Medical School researchers based at Massachusetts General Hospital. They included a study population of 1289 symptomatic adult patients positive for Covid-19 and document their blood group. The statistical analysis of the data determined that there is no correlation between blood type and intubation and/or death of the Covid-19 infected patients [9].

Though susceptibility of certain viral infections has been linked to antigenic determinants of ABO blood typing and severity of Covid-19 disease, the data from several studies remain inconsistent. This study aims to investigate the relationship between the blood group and risk of SARS-CoV-2 infection in 1050 covid-19 positive patients of Kaski district in Nepal. We hypothesize a link connecting ABO blood groups to SARS-CoV-2. We believe that this link will surely
help for a better understanding of the pathophysiology of Covid-19, as well as for the safety of the ongoing convalescent plasma therapy clinical trials.

2. Methods and Materials

The present study is carried out at Lifecare Diagnostics and Research Center in Pokhara. The research study started from 28th March 2021 until 25th October, 2021. The research proposal was approved by the ethical code of the Institutional Review Committee. The sample population of the study went with RT-PCR test from the nasopharyngeal, and oropharyngeal swab. This sample population was obtained after excluding the populations associated with comorbidities. The verbal interview helped collect information about the existing symptoms after the Covid-19 negative report, prior medical conditions, medication, cigarette/alcohol consumption habits, and the psycho-social context of the population. Moreover, all of the population knew about their blood group, so separate blood group determination was not needed. In case of old population, someone in the family (son, daughter or daughter-in law) carried them to the hospital and were ready to share the information on blood group and other details.

Random cluster sampling was used to collect data to ensure the participation of the population aged 20 to 65 years. The anonymity of the participants was maintained by assigning them unique identification numbers. The questionnaire and methodology were uniforms throughout the study in all the populations to maintain the same level of complexity and reduce the chances of confounding the results. The BMI was also calculated. SPSS version 20.0 was used for the statistical analysis of the data and to identify the association between blood type and Covid-19 severity.

3. Results

A total of 1150 patients were Covid-19 positive with asymptomatic, mild, moderate, and severe symptoms. Out of these, 100 samples were excluded from our study as we identified comorbidities along with Covid-19. Among 100 excluded sample, 60 were old population, 34 were adult and 6 were young population. The rest 1050 samples were from Brahmins, Chhetri, Gurungs, Magars and Dalits. The majority of the population were above 20 years and below 70 years. Besides Covid-19 test, these participants also underwent one to one interview questionnaires through phone to understand about their previous if any. Male who came to test for Covid-19 were found positive nearly twice than female diagnosed with Covid-19. Chi-square test was run for the data analysis. We identified that there is an association between Covid-19 disease severity and blood type. The calculated chi-square value in our study is 80.43 while the chi-square tabulated value is 32.671. Here, chi-square calculated > chi-square tabulated or chi-square critical value. The p value we have received is 0.0000021 which is < than 0.05. This means we reject our null hypothesis and accept the alternate hypothesis that there is an association between blood type and Covid-19 disease severity.
Our finding further shows that individuals with blood types B and O with Rh positive were more likely to test positive for Covid-19. However, individuals with blood type B+ and A+ were found having more severe symptoms in comparison to O+ blood type. Our study further reveals that an individual with rhesus negative (Rh−) blood groups may have a lower risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and severe coronavirus disease 2019 (Covid-19) illness. Please refer to Table 1 and Table 2 where Table 1 presented blood group and Covid-19 symptoms severity while Table 2 represented the chi-square expected value for blood group and symptoms severity.

Degree of Freedom = (columns − 1) * (rows − 1) = 7 * 3 = 21;
chi-square tabular value from the table = 32.671;
chi square calculated value = 80.43.

We have our chi-square calculated > chi-square tabulated or chi-square critical value as presented in Table 3, calculation of chi-square.

Table 1. Blood group and Covid-19 symptoms severity.

| Blood Group | Asymptomatic | Mild | Moderate | Severe | Total |
|-------------|--------------|------|----------|--------|-------|
| B+          | 25           | 77   | 85       | 136    | 323   |
| B−          | 0            | 8    | 12       | 18     | 38    |
| O+          | 30           | 134  | 99       | 73     | 336   |
| O−          | 4            | 6    | 9        | 7      | 26    |
| A+          | 22           | 32   | 57       | 78     | 189   |
| A−          | 1            | 1    | 2        | 1      | 5     |
| AB+         | 20           | 18   | 27       | 39     | 104   |
| AB−         | 4            | 6    | 11       | 8      | 29    |
| **Total**   | **106**      | **282** | **302** | **360** | **1050** |

Table 2. Chi-Square expected value for blood group and symptoms severity.

| Blood Group | Asymptomatic | Mild | Moderate | Severe | Total |
|-------------|--------------|------|----------|--------|-------|
| B+          | 32.608       | 86.749 | 92.901 | 110.743 | 323.000 |
| B−          | 3.836        | 10.206 | 10.930 | 13.029 | 38.000 |
| O+          | 33.920       | 90.240 | 96.640 | 115.200 | 336.000 |
| O−          | 2.625        | 6.983  | 7.478   | 8.914  | 26.000 |
| A+          | 19.080       | 50.760 | 54.360 | 64.800 | 189.000 |
| A−          | 0.505        | 1.343  | 1.438   | 1.714  | 5.000  |
| AB+         | 10.499       | 27.931 | 29.912 | 35.657 | 104.000 |
| AB−         | 2.928        | 7.789  | 8.341   | 9.943  | 29.000 |
| **Total**   | **106.000**  | **282.000** | **302.000** | **360.000** | **1050.000** |
### Table 3. Calculation of chi square.

| Observed (O) | Expected (E) | O − E | (O − E)^2 | (O − E)^2/E |
|--------------|--------------|-------|-----------|-------------|
| 25.00        | 32.61        | −7.61 | 57.88     | 1.77        |
| 0.00         | 3.84         | −3.84 | 14.72     | 3.84        |
| 30.00        | 33.92        | −3.92 | 15.37     | 0.45        |
| 4.00         | 2.62         | 1.38  | 1.89      | 0.72        |
| 22.00        | 19.08        | 2.92  | 8.53      | 0.45        |
| 1.00         | 0.50         | 0.50  | 0.25      | 0.49        |
| 20.00        | 10.50        | 9.50  | 90.27     | 8.60        |
| 4.00         | 2.93         | 1.07  | 1.15      | 0.39        |
| 77.00        | 86.75        | −9.75 | 95.03     | 1.10        |
| 8.00         | 10.21        | −2.21 | 4.87      | 0.48        |
| 134.00       | 90.24        | 43.76 | 1914.94   | 21.22       |
| 6.00         | 6.98         | −0.98 | 0.97      | 0.14        |
| 32.00        | 50.76        | −18.76| 351.94    | 6.93        |
| 1.00         | 1.34         | −0.34 | 0.12      | 0.09        |
| 18.00        | 27.93        | −9.93 | 98.63     | 3.53        |
| 6.00         | 7.79         | −1.79 | 3.20      | 0.41        |
| 85.00        | 92.90        | −7.90 | 62.43     | 0.67        |
| 12.00        | 10.93        | 1.07  | 1.15      | 0.10        |
| 99.00        | 96.64        | 2.36  | 5.57      | 0.06        |
| 9.00         | 7.48         | 1.52  | 2.32      | 0.31        |
| 57.00        | 54.36        | 2.64  | 6.97      | 0.13        |
| 2.00         | 1.44         | 0.56  | 0.32      | 0.22        |
| 27.00        | 29.91        | −2.91 | 8.48      | 0.28        |
| 11.00        | 8.34         | 2.66  | 7.07      | 0.85        |
| 136.00       | 110.74       | 25.26 | 637.92    | 5.76        |
| 18.00        | 13.03        | 4.97  | 24.72     | 1.90        |
| 73.00        | 115.20       | −42.20| 1780.84   | 15.46       |
| 7.00         | 8.91         | −1.91 | 3.66      | 0.41        |
| 78.00        | 64.80        | 13.20 | 174.24    | 2.69        |
| 1.00         | 1.71         | −0.71 | 0.51      | 0.30        |
| 39.00        | 35.66        | 3.34  | 11.17     | 0.31        |
| 8.00         | 9.94         | −1.94 | 3.77      | 0.38        |

**80.43**

This means we reject our null hypothesis and accept the alternate hypothesis that there is a significant relationship between Blood Group and Covid-19 Posi-
tive patients

For Individuals with comorbidities, we have excluded those sample from our study after identifying that certain blood group type (sample 100) are removed from the study. Those blood groups with associated comorbidities are listed in Table 4.

Populations having Covid-19 positive along with other comorbidities were identified and excluded from the study group to reduce the confounding factor (Graph 1 and Graph 2).

Table 5 illustrates that people with B+ blood group are 67% more likely to have the risk of having Covid-19 symptoms severity compared to the other blood groups. We are 95% confident that the relative risk of having severe symptoms in B+ blood group compared to other blood groups is between 0.65 to 0.70. The null value is 1. Since the 95% confidence interval does not include the null value (RR = 1), the finding is statistically significant.

Table 4. Individuals with blood group type and comorbidities associated.

| Blood Group | Comorbidities Associated |
|-------------|--------------------------|
| B+          | 37                       |
| B−          | 29                       |
| O+          | 20                       |
| O−          | 0                        |
| A+          | 7                        |
| A−          | 0                        |
| AB+         | 5                        |
| AB−         | 2                        |
| **Total**   | **100**                  |

Graph 1. Presented the graph plot when the degree of freedom is 21. Y-axis represents population while X-axis represents chi-square distribution plot.
Graph 2. Illustrate the degree of freedom where Y-axis represents p value while X-axis represents chi-square distribution plot.

Table 5. Identifying the relative risk or risk ratio and odd ratio.

|                      | Severe symptoms | Not severe | Total  |
|----------------------|-----------------|------------|--------|
| B+ blood group       | 136             | 187        | 323    |
| Other blood group    | 224             | 665        | 889    |

4. Discussion

It is crucial to better understand Covid-19, given the current pandemic’s death toll. We investigated whether blood type is relevant for Covid-19 severity. Overall, we found modest but consistent differences between blood types and Covid-19 risk. Individuals with blood group O+ were a major population presented for the RT-PCR test. This could be because the majority of the population in Nepal have O+ blood groups. For evidence, research titled ABO and Rh Blood Groups and their Ethnic Distribution in a Teaching Hospital of Kathmandu, Nepal has clearly mentioned that blood group O+ were found in the majority of the population (around 4755 which is 35.1%) [10].

Patients with blood type O+ and B+ are found with increased numbers of Covid-19 positive tests, however, sample populations with blood group B+ are at severe Covid-19 symptoms. Majority of O+ blood groups with Covid-19 positive tests had mild symptoms. AB blood group is the rarest blood group we found in our sample. We also found coherent evidence for protective associations between Rh-negative blood groups and SARS-CoV-2 infection. People having RH negative blood groups are found less likely to have Covid-19. However, given the relative rarity of rhesus negative blood types in our sample population, we cannot conclude this given our patient numbers in this study. Beside the medical report, a phone interview was also conducted where Individuals were asked about their blood type along with the disease severity and symptoms, hospital
admission, current situation, dietary habits, comorbidities, family and society acceptance etc. Counselling, mental health support, positive attitude, motivation was also provided to patients who were extremely fearful about the disease.

5. Limitation of the Study

The major limitation of the study is having small sample size and therefore could not generalize our findings. Moreover, the duration of the study is less than a year which is why we were unable to go back and follow up those participants.

6. Conclusion

This study found an association between blood type and Covid-19 disease severity. Majority of the population with blood group O+ were tested for Covid-19 through RT-PCR. Blood type B+ are found having severe Covid-19 symptoms. After blood group B+, the largest number of the population with A+ blood type was found having severe symptoms. Blood group O+, which was the highest number in the test, had only 6.95 percent of populations with severe symptoms. The present research study further found that there is a protective association between Rh-negative blood groups and SARS-CoV-2 infection. However, further study with bigger sample size and longer study duration is indeed vital for the generalization of the findings.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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