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# Correlation between ABO Blood Group typing and Severity of COVID-19 Cases in Ghadamis City, Libya

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**Abstract**

**Objectives:** This study examined the correlation between the ABO blood group system, Rh factors, and the susceptibility and severity of COVID-19. It also analyzed blood parameters and their association with disease outcomes .

**Materials & Methods:** A total of 286 nasopharyngeal swabs were collected following WHO guidelines for SARS-CoV-2 RNA detection. RNA was extracted using three kits: QIAamp, Viral RNA (QIAGEN), and the Mini kit. Reverse transcription polymerase chain reaction (RT-PCR) confirmed positive cases, and the Phoenix Dx 2019-nCoV kit was used for real-time PCR.

**Results:** Of the 286 patients, 153 (53.50%) were female, and 133 (46.50%) were male. COVID-19 was more common in females across all ABO blood groups (A: 52.4%, AB: 52%, B: 50%, O: 59.7%), with blood group A being the most prevalent. Severe cases were mostly blood group A (45.6%), while group O accounted for only 0.68%. In moderate cases, blood group B was predominant (44.7%). Rh-positive individuals were more prone to severe infection. Patients with severe disease showed lower hematological parameters, including hemoglobin, lymphocytes, white blood cells, red blood cells, and platelets, while moderate cases had higher white blood cell counts. Lymphopenia was prevalent in all COVID-19 patients.

**Conclusion:** This study highlights the relationship between ABO blood groups and COVID-19 severity, identifying comorbidities as significant contributors to mortality, particularly in Ghadamis City, Libya. Furthermore, the study provides an overview of the health status of COVID-19 patients in our region.

**KEY WORDS:** Novel coronavirus (SARS-CoV-2), COVID-19, infection susceptibility, lymphocytes (LYM), white blood cells (WBC)

## INTRODUCTION

Coronavirus disease 2019 (COVID-19), a highly transmissible respiratory disease, was first identified in December 2019 during an investigation into an outbreak in Wuhan, China [1]. Since its emergence, it has caused a global pandemic, with a high mortality rate resulting in over 3.4 million deaths worldwide [2]. The number of confirmed cases and deaths continues to rise rapidly, impacting numerous African countries, including South Africa, which has reported the highest number of cases in the WHO African region, with a total of 64,388 [3, 4].

Libya, the second-largest country in Africa, rich in natural resources and bordering the Mediterranean Sea, has also recorded the highest number of confirmed cases and deaths from COVID-19 globally[5].

Many studies reported an association between the ABO blood group and COVID-19 [6, 7]. They confirmed that group A person would be at the higher risk of contracting the infection, while group O people be at a lesser risk. The ABO system is one of the most important systems listed by the international society of blood transfusion, where it divides blood into four groups: A, B, AB, and O [8].

Blood groups are determined by carbohydrates present on the surface of red blood cells (RBCs), giving them the ability to act as receptors for different microorganisms and toxins [9]. Zhao et al. [6] reported the first suggestion of a link between ABO blood group and clinical COVID-19 manifestations. Subsequently, numerous studies have addressed this correlation [10]. Moreover, the Rhesus (RH) factor which is another RBC surface protein is suspected to have a role in the COVID-19 infection [11].

Recent researches have examined the associations between blood groups with COVID-19 infections worldwide [10, 12{Majeed, 2021 #14]}. Guidelines for disease management may benefit from an understanding of the correlations between blood group types and COVID-19 severity. Nevertheless, the relationships between the Rh and ABO blood systems may vary throughout populations [13]. Therefore, we carried out the current study to examine the association between the ABO blood group system with the susceptibility to, and severity of, COVID-19 infection; Additionally, to investigate the correlation between ABO blood groups and RH factor with the outcome of COVID-19 infection and evaluation of blood parameters of COVID-19 patients in Ghadamis, Libya.

## MATERIAL AND METHODS

A cross-section study conducted in Ghadamis City, Western Libya, during the period between August 2020 to 2021. A total of patients were recruited including (inclusion criteria: age, gender, laboratory findings of blood groups, severity of the illness hospitalization symptoms, etc.....

According to the WHO guides for nasopharyngeal swab collection, a Dacron polyester cotton swab and universal transport media (UTM) were used to collect the nasopharyngeal samples from study subjects to detect SARS COV-2 RNA. The viral RNA was extracted from the samples using QIA amp Viral RNA (QIAGEN)

Mini kit. The samples were either fresh or frozen; all the previous procedures were performed according to the manufacturer's instructions.

2019-nCoV RNA or genome was detected by revere transcription polymerase chain reaction (RT-PCR), the samples were performed using Phoenix Dx 2019-nCoV for qualitative RT-PCR-based assays. in respiratory specimens and sera with real-time PCR (Rotor-gene – QIAGENE). the primers and probe sequences of genes used RT-PCR for COVID-19 detection are provided in table 1. The kits follow CDC's and WHO's latest detection guidelines.

**Table 1:** Primers and probe sequences used in RT-PCR of COVID-19 detection

| Assay/ use | Oligonucleotide ID | Sequence (5`-3`) |
|---|---|---|
| RdRP | RdRP_SARSr-F2 | GTGARATGGTCATGTGTGGCGG |
| | RdRP_SARSr-R1 | CARATGTTAAASACACTATTAGCATA |
| | RdRP_SARSr-P2 | FAM-CAGGTGGAACCTCATCAGGAGATGCBBQ |
| | RdRP_SARSr-P1 | FAMCCAGGTGGWACRTCATCMGGTGATGCBBQ |
| E gene | E_Sarbeco_F1 | ACAGGTACGTTAATAGTTAATAGCGT |
| | E_Sarbeco_R2 | ATATTGCAGCAGTACGCACACA |
| | E_Sarbeco_P1 | FAM-ACACTAGCCATCCTTACTGCGCTTCGBBQ |

Standard venous blood was collected in Ethylene diamine tetra acetic acid (EDTA) tube for the analysis of blood groups and leukocytes. Serological (Antigen-antibody) reaction technique was used to identify the blood groups also the total and differential leucocyte count were estimated -parts full-automated hematological analyzer for complete blood count (CBC), Mindray®5150.

The investigation of the differences significant in the association between the ABO blood groups and RH factor with the susceptibility to severity of COVID-19 infection was studied by a Pearson's Chi-square test using by SPSS version 19 software (IBM Corporation, Somers, NY). The level of statistical significance was set at $P< 0.05$.

## RESULTS

A total of 286 Covid-19 patients were studied, comprising. 153 females (53.50 %) and 133 males (46.50%). We investigated the potential susceptibility of individuals with different ABO blood types to COVID-19 (Table 1). The distribution of ABO blood groups was as follows: 82 patients (28.7%), with blood

group A, 82 patients (28.1%) with blood group B, 50 patients (17.4%) with blood group AB, and 72 patients (25.2%) with blood group O.

The study aimed to assess the vulnerability of individuals with different ABO blood types to COVID-19. Table- 1 displays the distribution of the ABO blood types and the degree of the severity of COVID-19symptoms. Blood group A was the most represented among patients, with 45.6% (67/147) of those with severe COVID-19 belonging to this group, while blood group O accounted for 30.6% (1/147) of severe cases. In contrast, among patients with moderate COVID-19, blood group B was predominant, representing 44.7% (34/76) of cases. These results suggest that all ABO blood groups are susceptible to severe COVID-19 infection (Table 2).

**Table 2:** The correlation between ABO Blood group and degree of symptom of Covid-19.

| **ABO Blood group** | **Degree of symptoms** | | | **Total** |
|---|---|---|---|---|
| | **Moderates** | **No symptoms** | **Sever** | |
| **A** | 14 (17.1%) | 1(1.2%) | 67(81.7%) | 82 (28.7) |
| **AB** | 14(28%) | 2(4%) | 34(68%) | 50 (17.4) |
| **B** | 34(41.5%) | 3(3.7%) | 45(54.8%) | 82 (28.7) |
| **O** | 14(19.4%) | 57(79.2%) | 1(1.4%) | 72 (25.2) |
| **Total** | 76(26.6%) | 63(22%) | 147(51.4%) | 286 (100%) |

The presence of certain concomitant influences the course and progression of COVID-19. Our objective was to identify the comorbidities contributing to mortality in critically infected patients diagnosed with COVID-19. Certain individuals diagnosed with COVID-19experienced respiratory diseases, cancer, cardiovascular diseases, kidney failure and rheumatism and in some cases resulting in death (Table 3). Cardiovascular diseases, respiratory diseases and cancer were the most common comorbidities in with COVID-19patients. Cardiovascular failure was observed in 13 patients, and respiratory diseases in one patient, both of which contributed to mortality.

**Table 3:** Relationship between diseases and death cases.

| **Diseases** | **Death case** | |
|---|---|---|
| | **Live** | **Dead** |
| Respiratory diseases | 54 | 1 |
| Cancer | 14 | 0 |
| Cardiovascular diseases | 121 | 13 |

| | | |
|---|---|---|
| Kidney failure | 5 | 0 |
| Pregnancy | 1 | 0 |
| Rheumatism | 1 | 0 |
| No | 76 | 0 |
| **Total** | 272 | 14 |

The study also investigated the correlation between gender and ABO blood group in COVID-19 patients. Overall, the findings indicated a higher percentage of COVID-19 infection in females across all ABO blood groups (A, 52.4%/AB, 52%/B, 50%/and O, 59.7%). Conversely, the percentage was lower among males, thought the differences were not reach statistically significant (P > 0.532) (Table 4).

**Table 4:** Relationship between gender and ABO blood groups

| **Gender** | **ABO Blood group** | | | |
|---|---|---|---|---|
| | A | AB | B | O |
| Female | 43(52.4%) | 26(52%) | 41(50%) | 43(59.7%) |
| Male | 39(47.5%) | 24(48%) | 41(50%) | 29(40.2%) |
| **Total** | 82 | 50 | 82 | 72 |

*P* value > 0.532

Additionally, the results showed that male patients with COVID-19 exhibited more severe symptoms than female patients, although this difference was not statistically significant (P > 0.525) (Table 5).

**Table 5:** Relationship between gender and degree of symptoms

| **Gender** | **Degree of symptoms** | | |
|---|---|---|---|
| | Moderates | No | Sever |
| Females | 44 | 35 | 74 |
| Males | 32 | 28 | 73 |
| **Total** | 76 | 63 | 147 |

*P* value > 0.525

The ABO and Rh blood grouping systems are non-modifiable risk factors that play important role in the susceptibility, severity and outcomes of COVID-19 infection. Our results suggest that blood group O may

provide a protective effect against infection, while blood group A is associated with a higher risk of severe disease.

Rh-positive individuals were more prone to severe infections and complications compared to Rh-negative individuals. Regarding the Rh factor, Rh-negative individuals had a lower risk of initial infection compared to Rh-positive individuals. The RH factor also played a role in the severity COVID-19 infection, although the difference was not statistically significant (P > 0.730) (table 6).

**Table 6:** The frequency of Rhesus factor (Rh) in ABO blood group

| **Rhesus factor** | **ABO Blood group** | | | |
|---|---|---|---|---|
| | A | AB | B | O |
| Rh (+) | 46 | 30 | 52 | 46 |
| Rh (-) | 36 | 20 | 30 | 26 |
| **Total** | 82 | 50 | 82 | 72 |

*P* value > 0.730

Globally, the prevalence of COVID-19 patients has increased, making it crucial to evaluate hematological variables when assessing the outbreak. The results showed that severe COVID-19 patients exhibited lower hematological parameters whereas moderate COVID-19 patients hadhigher lymphocytes (LYM), red blood cells (RBC) and platelets (PLT). In severe COVID-19cases, the mean ± SD values of the hematological parameters were as follows: hemoglobin (HB) 11.00 ± 0.86, lymphocytes 0.97± 0.33, white blood cells (WBC) 5.68± 0.98, red blood cells 4.63± 0.46 and platelets 243.68± 33.82 (Table 7). White blood cell levels were significantly higher in moderate cases compared to severe COVID-19 cases, with statistically significant. Patients with COVID-19 frequently experienced lymphopenia,distinguishing it from typical viral infections, which usually show elevated lymphocyte counts but rarely eosinopenia. The pathophysiology of COVID-19 is believed to be supported by the inflammatory response.

**Table 7:** Degree of symptoms with hematological markers

| | Degree of symptom | | | | | | | | | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Moderate | | | No | | | Sever | | | |
| | N | Mean | SD | N | Mean | SD | N | Mean | SD | |
| LYM | 61 | 1.94 | 0.40 | 75 | 1.27 | 0.47 | 149 | 0.97 | 0.33 | 0.000 |
| WBC | | 5.80 | 0.79 | | 5.31 | 0.76 | | 5.68 | 0.98 | 0.000 |
| HB | | 11.88 | 0.78 | | 11.22 | 0.63 | | 11.00 | 0.86 | 0.400 |
| RBC | | 5.15 | 0.51 | | 5.47 | 5.58 | | 4.63 | 0.46 | 0.520 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| PLT | | 310.68 | 36.76 | | 260.94 | 43.22 | | 243.68 | 33.82 | 0.000 |

The comparison of hematological markers with symptom severity showed that LYM, WBC and PLT levels were significantly higher in patients with moderate symptoms compared to those sever symptoms (Table 7).

## DISCUSSION

In Libya, coronavirus disease 2019 (COVID-19) disease still causes a major public health threat leading to considerable fatalities and impacting global health systems. On a global scale, there have been 440,807,756 confirmed COVID-19 cases, resulting in 5,978,096 deaths. The cases in Libya reached 497,958 with 6,299 deaths according to the National Centre for Disease Control of Libya [14].

Multiple factors contribute to the severity, complications and mortality risk associated with COVID-19, and among these factors is blood groups. The study was performed on 286 patients spanning an age range from one to 90 years admitted to the intensive care unit (ICU) of Ghadamis Hospital between August 2021 and August 2022. All cases were positive for COVID-19.

In this study, we confirmed the relationship between ABO blood groups and COVID-19 susceptibility in patients infected. Blood group A individuals had a significantly higher risk of acquiring COVID-19 compared with other blood groups and blood group O had a significantly lower risk for the infection compared with non-O blood groups. These results agree with several studies [6, 15].

The association between the ABO blood groups and the vulnerability to contracting COVID-19 infection was confirmed within the different blood groups. ABO blood groups exhibited associations with the exhibited associations severity and mortality of COVID-19 among patient's groups. Consequently, blood groups A and O are two crucial factors to be considered when evaluating the prognosis of patients with COVID-19.

Several studies reported the possible association between the ABO blood groups and the susceptibility to and severity of COVID-19 disease. However, some studies have shown conflicting findings. When the association of ABO blood group with susceptibility to COVID-19 was analyzed from the perspective of ABO antibodies instead of ABO blood group antigens, the same conclusions were reached. Referring to the published series of patients published by Zhao et al. [6] it was observed that subjects with anti-A in serum (i.e. blood groups B and O) were significantly less represented in the COVID-19 group than those lacking anti-A whatever the blood group, whereas there was no significant difference vs circulating anti-B [16].

Various complications and risk factors have been identified and associated with the progression of COVID-19 into a severe and moderate stage, including cardiovascular diseases, chronic lung diseases, diabetes, hypertension, tumors, liver and kidney diseases, immunodeficiency, and pregnancy, in addition to old age, male sex, obesity, and smoking [17].

The study conducted by Bshaena et al. [14] demonstrated variations in the prevalence of the ABO and Rh systems may differ among populations, and their linkage with the COVID-19 may differ too. Moreover, in

concordance with the results observed in our study, their findings indicated a significantly higher prevalence of blood group A among patients with severe COVID-19 compared to those with non-severe cases, while the O blood group exhibited a higher prevalence in non-severe COVID-19 cases compared to severe cases.

Due to variations in the prevalence of the ABO and Rh blood groups in different patients, their association with COVID-19 may exhibit variability. Hence, an investigation into the relationship between the ABO blood groups and Rh was conducted. Our findings showed that no significant association was found between Rh and susceptibility/severity of the disease. These results agree with the findings reported by [18].

In our results, the occurrence of COVID-19 was notable in the in age group 51-70 years, showing a higher severity of the disease. Many studies published results of the effect of age on COVID-19 disease severity, that is, the direct effect of age after accounting for important age-related risk factors such as diabetes, cardiovascular disease and chronic pulmonary disease. The result was that the effect of age was rather small [19].

The extensive occurrence of COVID-19 has a substantial effect on both blood coagulation and the hematological system. Common hematological findings related to the severity of COVID-19 include decreased lymphocytes, increased white blood cell count, higher neutrophils, and enhanced blood clotting tendencies [20].

Regarding the comparison of hematological parameters, LYM, WBC, HB, RBC and PLT revealed that the moderate categories were higher than the severe group with statistically significant. This is in agreement with these findings in China, they reported that Lym, RBC, and HGB were significantly higher in the moderate group than in the severe group ($P<0.05$). While WBC and Neu were higher the severe patients than in moderate patients with statistically significant ($P<0.05$). Furthermore, they mentioned that no significant difference in platelet count between the moderate and severe groups [21]. In a Brazilian study, they mentioned that distinguishing between severe and non-severe cases in COVID-19 patients is achievable through baseline WBC counts and comprehensive cell population data (CPD) measures. This is evident in the higher baseline WBC and NEU counts observed in individuals with COVID-19-positive testing who were subsequently hospitalized [22].

In the present study, Patients with COVID-19 often suffered from lymphopenia, particularly those who exhibited elevated (WBC) counts. Wang et al. [21] explained the considerable lymphopenia in severe cases might be due to the SARS-CoV-2 proceeds to infiltrate more lymphocytes, proliferating and leading to the demise or depletion the lymphocytes upon reaching the spleen and other immune organs. Many studies have confirmed this lymphopenia [21-23].

## CONCLUSION

In summary, we reported the associations between COVID-19 severity and susceptibility in ABO blood groups. Patients with the type A blood group are more likely to develop severe COVID-19 infection, whereas patients with type O blood group have a lower risk of developing severe COVID-19 infection. In addition, several comorbidities cause mortality in critically infected patients diagnosed with COVID-19. Blood type A individual may require heightened personal protection to lower their risk of contracting an infection and more

surveillance. This study demonstrated that the hematological parameters can be utilized as a marker to differentiate between moderate and severe COVID-19 cases. This data will help clinicians to predict the severity and disease classification of patients to choose proper treatment and use this study as a clinical guide.

**Declarations:**

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**Competing interests:** The authors declare that they have no conflicts of interest.

**Ethical clearance:** Not applicable

**Data availability:** The datasets generated during this study are available in this manuscript.

**Authors' Contributions:** Siham Agouri drafted the manuscript, Asma Elramli designed the study, collected the data, analyzed and interpreted the data, Ghassan Tayh contributed to final writing and editing the manuscript. Elmundr Abughnia helped in performing the experimental part of the manuscript. Hafsa Alemam designed, supervised the study and interpreted the data. Naila Abdulrahman and Abdulghafar Shihab collected samples. Ibrik ashour performed the statistical part and interpreted of the results. Salem Ali Bozrayda supervised the study.

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