

THE EVALUATION OF PREVALENCE AND HEMATOLOGICAL PARAMETERS FOR ASSESSMENT OF SEVERITY OF CORONAVIRUS INFECTION

Abstract

Objectives: We determined the prevalence of coronavirus (COVID-19 virus) infection on the basis of age, sex, clinical presentation and risk of ABO Blood Groups antigens with COVID-19 virus. We also highlight the Hematological Parameters such as neutrophils to lymphocytes ratio and thrombocytopenia to assess severity of COVID-19 viral infection.

Methodology: A descriptive study was conducted from May 2020 to May 2021 at rural and urban areas of District Shaheed Benazir Abad in the homes of patients diagnosed with Covid-19 viral infection where they were present in quarantine. A total of 351 patients with their ages ranging between 12 and 68 including 329 males and 21 females present in isolation within their homes with diagnosis of coronavirus infection were included in this study. The clinical features of the patients were noted, and 5 ml of blood was collected in an EDTA bottle from each patient for determination of blood group and complete blood account at the diagnostic and research pathology laboratory of PUMHS, Nawab Shah.

Results: The mean age of these patients was 40 ± 28 years while male to female ratio was 15.6:1. Out of the 351 patients, 169 (45.3%) were blood group A, 117 (33.8%) were blood group B, 50 (11.3%) were blood group AB and 26 (8.6%) were blood group O. The mean value of neutrophil to lymphocyte ratio was found to be 4.7 ± 1.7 while the mean value of platelets count was $210000 \pm 95000/\text{mm}^3$ in these patients.

Conclusion: We concluded that the ratio of coronavirus infection among the patients with Blood Group A was higher than the B, AB and O. However, the study revealed a significantly higher proportion of AB+ male patients as compared to females who were infected with COVID-19.

Keyword: Prevalence, Coronavirus Infection (COVID-19), Blood Groups, hematological parameters Assessment.

INTRODUCTION

The word corona is derived from Italian language that means crown, therefore spike-like glycoprotein on the surface of the virus gives rise crown appearance to the corona virus so it is called corona virus that belongs to be family of coronaviridae and sub-family coronaviridae divided into alpha-beta, Gamma and Delta coronavirus¹. Corona Viruses are enveloped viruses with a large plus-strand RNA genome being 27-32kb in size capped and polyadenylated and range from 80 to 160 nm in diameter. The genome of coronavirus composed of 5 and 3 untranslated region (UTR) and open reading frame (ORF) la/b structural genes present at 3 terminus encodes for the structural proteins including spike (s) envelope (E) membrane (m) and nucleocapsid which are common features to all Coronavirus². When the corona virus transmitted into the human or animal, the lifecycle of the virus is started and the ACE2 receptor is an intra-membrane receptor on the II pneumocystis, where the virus is able to replicate after getting endocytosis with in the cytoplasm as the viral load increases within the alveolar epithelial that will burst releasing the newly replicated viral RNA³. Then the virus enters into another alveolar epithelial cells causes wide destruction of cells and set up the inflammatory reaction with engorgement of blood vessels and release of inflammatory mediators by the damaged epithelial cells that causes neutrophils, macrophages and lymphocytes along with inflammatory exudate that accumulated in alveolar spaces of lungs leading into consolidation or pneumonia⁴. The inflammation within the lung progress to systemic inflammatory response syndrome and the patient with covid 19 will develop symptoms and the incubation period from onset of symptoms of disease to death ranged from 6 to 41 days and during this period, patients present with Fever, dry cough, shortness of breath, myalgia of fatigue, nasal congestion, chills, night sweats, headache, rhinorrhea, chest pain, nausea and vomiting⁵. Accurate molecular diagnostic tests such as polychromatic chain reaction for detection of ribonucleic acid (R N A) of covid 19 is necessary for confirming a diagnosis of coronavirus disease.⁶ Individuals with blood group A are more liable to contract COVID infection than others owing to the owing to the receptor binding domain of SARS-CoV-2 sequence similarity percent on the blood group A persons of respiratory epithelial cells, leading to SARS-CoV-2 infection in these person^{7,8}. The common hematological abnormalities in COVID-19 infection include neutrophilia and lymphopenia. Neutrophilia is caused by the release of neutrophil-chemoattractant elements and the resulting recruitment of neutrophils is a host response to viral infection.⁹ The COVID-19 infection causes lymphopenia due to the exaggerated inflammatory response known as cytokine storm that is composed of interleukin (IL)-2, IL-7, interferon- γ and tumor necrosis factor- α that may promote destruction of the lymphocytes¹⁰.

Thus, considering the evidence hinting at the susceptibility of individuals with specific hematological characteristics, we undertook the current study to evaluate association of blood group antigens with COVID infection. We also highlighted the hematological parameters of COVID-19 infection to assess the severity of disease.

METHODS AND MATERIALS

Study Design: A descriptive study was undertaken.

Study Setting and Duration: The study was conducted from May 2020 to May 2021 at rural and urban areas of District Shaheed Benazir Abad in the homes of patients with Covid-19 virus where they were present in quarantine.

Inclusion Criteria: Total 351 patients with their ages ranging between 12 and 68 who were present in isolation within their homes with confirmed diagnosis of coronavirus infection were included in this study.

Exclusion Criteria: Patients with diabetes mellitus, hypertension, malaria, dengue fever, viral hepatitis, pregnant women, and patients who were not giving permission for sampling were excluded from this study.

Data Collection Procedure:

The clinical features of all these patients such as running nose, fever, dry cough, dyspnea and sore throat were noted. All these patients were already diagnosed by PCR test for coronavirus infections. 5 ml blood samples of the COVID-19 patients were received and out of each 5ml, 3ml was mixed in a bottle containing EDTA while 2ml blood was placed in a plain bottle for detection of blood group. The hematological parameters were detected by hematology analyzer (Nihon Kohden) and ESR was detected by Westergren method; ABO blood groups were done by forward and reverse methods. In the forward method the patient's saline-washed red cells were mixed with known commercially prepared antiserum of anti-A, anti B and anti-D in a test tube; the mixture was incubated at room temperature followed by centrifugation. For the reverse method, the patient's serum was mixed with reagent red cells of groups of A, B and O (available commercially), incubated at room temperature followed by centrifugation. Then a red cell button observed at the bottom of the tube was examined for agglutination.

Data Analysis: All data was analyzed using Microsoft excel software. All quantitative variables were presented as mean and standard deviation while the qualitative variables were presented as frequency and proportions.

RESULTS

The mean age of these patients was 40 ± 28 years while males were predominant in the study. The mean value of hemoglobin, erythrocytes sedimentation rate, total leukocyte count, differential leukocyte count such as percentages of neutrophils, lymphocytes, neutrophil to lymphocyte ratio and platelet count were 12.1 ± 0.1 gm/dl, 50 ± 10 /hr., $13000 \pm 1000/\text{mm}^3$, Neutrophils $80 \pm 5\%$, Lymphocyte $10 \pm 2\%$, 4.7 ± 1.7 , $210000 \pm 95000/\text{mm}^3$ respectively. These patients were mildly anemic and their ESR as well as total leukocytes counts high indicating infection. There was neutrophilia with lymphopenia and raised neutrophil to lymphocyte ratio. Out of a total of the 351 study participants, 169 (45.3%) were Blood Group A, 117 (33.8%) Blood Group B while 50 (11.3%) and 26 (8.6%) were Blood Groups AB and O respectively.

Table 1. DEMOGRAPHY OF CORONAVIRUS INFECTION N=351

Characteristics	Values
Age	40 ± 28
Gender	
Male	309 (88%)
Female	42 (12%)
Clinical Presentations	
Running nose/ Headache	341 (97.2%)
Fever and body aches	250 (71.2%)
Dry cough	210 (59.8%)

Dyspnea	115 (32.8%)
Sore throat	280 (79.77%)
Hematological Parameters	
Hemoglobin (gm/dl)	12.1 \pm 0.1
ESR (hours)	50 \pm 10
Total leukocyte count (mm ³)	13000 \pm 1000
Different leukocyte count	
Neutrophils	80 \pm 5 %
Lymphocyte	10 \pm 2 %
Neutrophil to lymphocyte ratio	4.7 \pm 1.7
Platelet count (mm ³)	210000 \pm 95000

The most common blood group was A positive. However, the study revealed a significantly higher proportion of AB+ male patients as compared to females who were infected with COVID-19 ($p=0.017$) as illustrated in Table 2. Moreover, O positive blood group was significantly more common in female patients than male patients.

TABLE 2. THE DISTRIBUTION OF BLOOD GROUPS WITH RESPECT TO GENDER

Blood groups	Total	Male	Female	p-value
A +	164 (46.7%)	137 (44.3%)	27 (64.3%)	0.017
B +	113 (32.2%)	105 (34%)	8 (19%)	
AB +	49 (14%)	47 (15.2%)	2 (4.8%)	
O +	25 (7.1%)	20 (6.5%)	5 (11.9%)	

DISCUSSION

The prevalence on the basis of age, sex, clinical presentation and hematological parameters as well as risk of blood groups antigens among the patients with COVID-19 infection were conducted by the international and local studies. According to World Health Organization report in July 2021, about 185 million people were infected by Coronavirus out of which 69.3 million people recovered, 4 million died, while 12 million people were active cases of coronavirus infection. The National command and control system reported that in Pakistan 966000 people were infected with coronavirus, 22460 died, 910000 people recovered and 34,000 active cases remained in Pakistan.¹¹

According to Huang, C. et al report in Wuhan city of China, COVID-19 caused viral pneumonia in 41 patients including 30 male and 11 female with their age ranged between 19 and 80 years diagnosed by PCR test, and they presented with fever, cough, myalgia and dyspnea.¹² They also reported that the disease is transmitted from humans and other mammals including camels and bats. Chen N et al noted Covid-19 virus infection such as pneumonia among the 99 cases diagnosed by PCR test including 67 male and 32 female with their ages ranging between 42 and 68 years present with similar clinical features reported in above study.¹³

Diagnosed Covid-19 infected cases of 452 patients including 279 males and 173 females with their age ranged between 44 and 72 years, admitted to five leading hospitals in different cities of the Pakistan, were included in the study conducted by Saadia Omer et al.¹⁴ They reported that these patients were suffering from fever, followed by cough, dyspnea, fatigue, myalgias, generalized body aches, vomiting, headache, diarrhea, sore throat, sputum, production, nausea, loss of taste, rhinorrhea, anosmia and nasal congestion. A total of 412 patients of more than 60%

male with their ages ranging between 41 and 42 years who were residents of East Karachi and tested positive for SARS-CoV-2 as reported by Shumaila T et al.¹⁵ recorded three most common symptoms such as fever, cough and running nose.

The study conducted by Noor A et al at Rawalpindi stated that ABO blood grouping was done by test tube method among the 326 patients infected with Covid-19 virus diagnosed by PCR test.

They noted that the blood group A was found to be 122 (37.4%) having significant association with COVID infection and mortality but blood group O had least prevalence 71 (21.8%) with COVID-19 infection.¹⁶

A total of 78 patients with a mean age 45 years including 41 females and 37 males was studied by Yang H.¹⁷ The neutrophilia with lymphopenia and higher neutrophil to lymphocytes ratio were detected in these patients with severe illness caused by Covid-19 infection. Similar studies conducted by various laboratory workers showed elevated levels of neutrophil to lymphocyte ratio among the older males of the Asian and Pakistan populations as compared to their younger age group.^{18,19} This ratio was used to assess severity of Covid-19 virus infection that is higher in the western population.²⁰ Forty-nine (27.5%) out of 178 patients were diagnosed with severe disease of Covid 19 infection, and 129 patients with non-severe disease and the severe disease groups had significantly lower platelet count as reported by Changqian Bao et al.²⁰

Strengths and Limitation

The current study revealed important information related to COVID-19 infection and adds substantially to the literature however, one limitation of our study was that it is a single center study and future studies should be from multiple centers so that a more diversified sample can be collected.

CONCLUSION

We concluded that the ratio of coronavirus infection among the patients with Blood Group A was higher than the B, AB and O. However, the study revealed a significantly higher proportion of AB+ male patients as compared to females who were infected with COVID-19. Hematological parameters such as neutrophil to lymphocyte ratio and thrombocytopenia were detected in few patients requiring emergency treatment. Screening of Covid infection would be necessary.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

References

1. Kadam SB, Sukhramani GS, Bishnoi P, Pable AA, Barvkar VT. SARS CoV 2, the pandemic coronavirus: Molecular and structural insights. *Journal of basic Microbiology*. 2021 Mar;61(3):180-202.
2. Singh J, Samal J, Kumar V, Sharma J, Agrawal U, Ehtesham NZ, Sundar D, Rahman SA, Hira S, Hasnain SE. Structure-function analyses of new SARS-CoV-2 variants B. 1.1. 7, B. 1.351 and B. 1.1. 28.1: clinical, diagnostic, therapeutic and public health implications. *Viruses*. 2021 Mar;13(3):439.
3. Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *The lancet*. 2020 Feb 15;395(10223):473-5.
4. Azhar H, Jasndeeep K, Elsa T, Salma T, SM TS. Novel COVID-19: A Comprehensive Review of Transmission, Manifestation, and Pathogenesis. *Cureus*. 2020;12(5).
5. Hanson KE, Caliendo AM, Arias CA, Englund JA, Lee MJ, Loeb M, Patel R, El Alayli A, Kalot MA, Falck-Ytter Y, Lavergne V. Infectious Diseases Society of America guidelines on the diagnosis of coronavirus disease 2019. *Clinical infectious diseases*. 2020 Jun 16.
6. Wu SC, Arthur CM, Wang J, Verkerke H, Josephson CD, Kalman D, Roback JD, Cummings RD, Stowell SR. The SARS-CoV-2 receptor-binding domain preferentially recognizes blood group A. *Blood advances*. 2021 Mar 9;5(5):1305.
7. Shibeel S, Khan A. ABO blood group association and COVID-19. COVID-19 susceptibility and severity: a review. *Hematology, Transfusion and Cell Therapy*. 2022 Mar 21;44:70-5.
8. Wang J, Li Q, Yin Y, Zhang Y, Cao Y, Lin X, Huang L, Hoffmann D, Lu M, Qiu Y. Excessive neutrophils and neutrophil extracellular traps in COVID-19. *Frontiers in immunology*. 2020;2063.
9. Fathi N, Rezaei N. Lymphopenia in COVID 19: Therapeutic opportunities. *Cell biology international*. 2020 Sep;44(9):1792-7.
10. Coronavirus N. situation reports-World Health Organization (WHO), 2019 Novel Coronavirus (2019-nCoV) in the US. US Centers for Disease Control and Prevention (CDC), symptoms of Novel Coronavirus (2019-nCoV)–CDC, china Travel Advisory-US State Department. Accessed. 2020 Jan;31.
11. National Command and Control Center Pakistan 2021
12. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet*. 2020 Feb 15;395(10223):497-506.
13. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Yu T. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The lancet*. 2020 Feb 15;395(10223):507-13.
14. Omer S, Sarwar MB, Roman M, Usman M, Khan MA, Afzal N, Qaiser TA, Yasir M, Shahzad F, Tahir R, Ayub S. Epidemiology, Clinico-Pathological Characteristics, and Comorbidities of SARS-CoV-2 infected Pakistani Patients. *medRxiv*. 2021 Jan 1.
15. Tahir S, Tahir SA, Arif TB, Majid B, Majid Z, Malik F, Ahmed A, Memon A, Ahmed J. Epidemiological and clinical features of SARS-CoV-2: a retrospective study from East Karachi, Pakistan. *Cureus*. 2020 Jun 17;12(6).

16. Noor A, Tashfeen S, Akhtar F, Anwar N, Din HU, Akhtar F. Association of COVID-19 with ABO blood groups in tertiary care center of Pakistan. PAFMJ. 2020 Jul 30;70(1):S251-55.
17. Yang H, Xu Y, Li Z, Yan L, Wang J, Liao P. The clinical implication of dynamic hematological parameters in COVID-19: A retrospective study in Chongqing, China. International journal of general medicine. 2021;14:4073.
18. Tahir S, Tahir SA, Arif TB, Majid B, Majid Z, Malik F, Ahmed A, Memon A, Ahmed J. Epidemiological and clinical features of SARS-CoV-2: a retrospective study from East Karachi, Pakistan. Cureus. 2020 Jun 17;12(6).
19. Zhao J, Yang Y, Huang H, Li D, Gu D, Lu X, Zhang Z, Liu L, Liu T, Liu Y, He Y. Relationship between the ABO blood group and the coronavirus disease 2019 (COVID-19) susceptibility. Clinical Infectious Diseases. 2021 Jul 15;73(2):328-31.
20. Bao C, Tao X, Cui W, Yi B, Pan T, Young KH, Qian W. SARS-CoV-2 induced thrombocytopenia as an important biomarker significantly correlated with abnormal coagulation function, increased intravascular blood clot risk and mortality in COVID-19 patients. Experimental Hematology & Oncology. 2020 Dec;9(1):1-8.