INTERNATIONAL ANATOLIA ACADEMIC ONLINE JOURNAL
e-ISSN 2148-3159 HEALTH SCIENCES RESEARCH ARTICLE

COMPARISON of LABORATORY FINDINGS and MORTALITY RATES of VARIANT ALPHA and SARS-COV-2 INFECTED PATIENTS

Dr. Adem Keskin, 0000-0003-1921-2583
Recai Aci, 0000-0002-3332-6619
Dr. Mehmet Hakan Taskin, 0000-0002-1374-7766
Dr. Mukadder Erdem, 0000-0001-7796-3671
Dr. Melek Bilgin, 0000-0003-0025-8717
Dr. Murat Ari, 0000-0002-1504-7050

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
03.10.2021	26.12.2021	30.12.2021

Correspondence: *Adem Keskin, Dr, Aydın Adnan Menderes University, Institute of Health Sciences, Department of Medical Biochemistry, Turkey, e-mail: ademkeskin78@gmail.com*

ABSTRACT

In our study, the common laboratuvary findings affecting SARS-CoV-2 patient outcomes were designed on the levels detected in Variant Alpha patients. 115 Variant Alpha inpatients, 115 SARS-CoV-2 inpatients and 111 healthy individuals were included in this study. Variant Alpha White Blood Cell (WBC), Platelet, Neutrophil, Lymphocyte, Monocyte count, Neutrophil Lymphocyte Ratio(NLR), Monocyte Lymphocyte Ratio (MLR), Hemoglobin, Procalcitonin, C-reactive protein (CRP), Ferritin, Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), International Normalized Ratio(INR), D-dimer levels and SARS-CoV-2 patients levels were compared. This comparison also includes comparison with the levels of healthy individuals. WBC, Neutrophil, MLR, NLR, PCT ($p=0.037$), Ferritin, CRP, aPTT, D-dimer levels of the Variant Alpha group were higher than the levels of SARS-CoV-2 group, and Lymphocyte ($p=0.001$), PT, INR levels of the Variant Alpha group were lower than the levels of SARS-CoV-2 group ($p<0.001$). There was no difference between the mortality rates observed in the Variant Alpha group and the SARS-CoV-2 group ($p>0.05$). In terms of laboratory findings, Variant Alpha patients experience more severe disease than SARS-CoV-2 patients. The reason that there was no difference in mortality rate between the groups may be the higher PT and INR values of the SARS-CoV-2 group.

Keywords: Variant Alpha, SARS-CoV-2, Mortality Rate, D-dimer, Prothrombin Time, C-reactive protein

VARYANT ALFA VE SARS-COV-2 ENFEKTE HASTALARIN LABORATUVAR BULGULARININ VE MORTALİTE ORANLARININ KARŞILAŞTIRILMASI

ÖZET

Çalışmamız, SARS-CoV-2 hasta sonuçlarını etkileyen yaygın laboratuvar bulgularının, Varyant Alfa hastalarında tespit edilen düzeyleri üzerine tasarlanmıştır. Bu çalışmaya 115 Varyant Alfa yatan hasta, 115 SARS-CoV-2 yatan hasta ve 111 sağlıklı birey dahil edildi. Varyant Alfa hastaların Beyaz Kan Hücresi (WBC), Trombosit, Nötrofil, Lenfosit, Monosit sayısı, Monosit Lenfosit Oranı (MLR), Nötrofil Lenfosit Oranı (NLR), Hemoglobin, Prokalsitonin, C-reaktif protein (CRP), Ferritin, Protrombin Zamanı (PT), Aktive Parsiyel Tromboplastin Zamanı (aPTT), D-dimer, International Normalized Ratio (INR) seviyeleri SARS-CoV-2 hasta

seviyeleri karşılaştırıldı. Bu karşılaştırma aynı zamanda sağlıklı bireylerin seviyeleri ile karşılaştırmayı da içerir. Varyant Alfa grubunun WBC, Nötrofil, MLR, NLR, PCT (p=0.037), Ferritin, CRP, aPTT, D-dimer seviyeleri SARS-CoV-2 grubunun seviyelerinden önemli ölçüde yüksekti (p=0.001). Ayrıca, Varyant Alfa grubunun Lenfosit, PT, INR seviyeleri SARS-CoV-2 grubunun seviyelerinden anlamlı derecede düşüktü (p<0.001). Varyant Alfa grubunda ve SARS-CoV-2 grubunda gözlemlenen ölüm oranları arasında anlamlı bir fark yoktu. Laboratuvar bulguları açısından Varyant Alfa hastaları SARS-CoV-2 hastalarına göre daha şiddetli hastalık yaşamaktadır. Gruplar arasında ölüm oranı farkı olmamasının nedeni, SARS-CoV-2 grubunun PT ve INR değerlerinin yüksek olması olabilir.

Anahtar Kelimeler: Varyant Alfa, SARS-CoV-2, Ölüm Oranı, D-dimer, Protrombin Zamanı, C-reaktif protein

INTRODUCTION

Public Health of England reported the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant of concern (VOC) lineage B.1.1.7, also referred to as VOC 202012/01 or 20I/501Y.V1 on 14 December 2020 (1). The World Health Organization renamed the variants SARS-CoV-2 on 15 June 2021. He named this British variant, Variant Alpha (2). Its defining characteristics include a deletion and several mutations within the key encoding the spike protein, remarkably Asparagine501Tryrosine in the receptor-binding domain. In addition, this impact of structural changes to the features of the spike protein has raised concern about transmissibility, pathogenicity, and efficacy of the variant on vaccine impact (3). Alpha is assumed to be 50% more contagious than the existing variants in a histogram showing simulated case incidence orbits of existing SARS-CoV-2 variants and Alpha variant (4).

SARS-CoV-2 stimulates pro-thrombotic changes. In addition, in SARS-CoV-2 infection, there is a correlation between significant increase in D-dimer levels, an indirect marker of thrombosis, and increase in the rate of complication (5). Common laboratory findings affecting SARS-CoV-2 patient outcomes were found to be White Blood Cell (WBC) levels and acute phase reactants. In addition, it was concluded that Neutrophil, Lymphocyte count, C-reactive protein (CRP) and D-dimer levels tended for a patient to have a mild to moderate disease course versus a severe course or eventual death (6).

In our study, the common laboratuvary findings affecting SARS-CoV-2 patient outcomes were designed on the levels detected in Variant Alpha patients. Variant Alpha patients' WBC, Platelet, Neutrophil, Lymphocyte, Monocyte count, Neutrophil Lymphocyte Ratio (NLR), Monocyte Lymphocyte Ratio (MLR), Hemoglobin, Procalcitonin (PCT), C-reactive protein (CRP), Ferritin, Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), International Normalized Ratio (INR), D-dimer levels and SARS-CoV-2 patients levels were compared. This comparison also includes comparison with the levels of healthy individuals.

MATERIAL and METHOD

Our study was conducted after obtaining ethics board approval of an Training and Research Hospital, dated 24.03.2021 and numbered GOKA/2021/6/4.

In our study, 115 patients with a mean age±standart deviation of 63.03±16.12, who were followed up in service and intensive care units (ICU) of a Training and Research Hospital between February 1, 2021 and April 1, 2021, who were found to be positive for SARS-CoV-2 in swab samples, 115 patients with a mean age±standart deviation of 59.30±15.50 who were found to be Variant Alpha positive in swab samples were included. In addition, 111 healthy individuals with a mean age±standart deviation of 54.72±19.90 were included as a control group.

Bio-Speedy, SARS-CoV-2 Double Gene RT-qPCR (Bioeksen, Istanbul, Turkey) kit was used to diagnose SARS-CoV-2 in patients and it was studied with BioRad CFX96 RT-PCR. SARS-CoV-2 was diagnosed with the SARS-CoV-2 nucleoprotein and oligoprimers of the ORF1ab conserved gene region with this kit. RNase P human mRNA gene region was used as internal control. Ct (Cycletreshold) ≤38 sigmoidal values were evaluated as positive for SARS-CoV-2 according to the manufacturer's quides. As stated in the studies of Washington NL et al. (7), in cases where the N gene is detected, since samples with Ct ≥27 could not produce strong amplification, positive samples with Ct <27 Bio-Speedy for the detection of SARS-CoV-2 Variant Alpha, SARS- COV-2 variant plus (Bioeksen, Istanbul, Turkey) was included in the mutation study with the kit. The Threshold value (CT) ≥ 27 and the samples that were not taken into mutation study were evaluated as SARS-COV-2 positive with this kit. Only one positive result for each patient was included in the study and positive results on repeat tests of a patient were excluded from the study.

Whole blood parameters, Hemoglobin, CRP, Ferritin, PCT, which are important biomarkers for the prognosis of the disease in the inflammatory process and Coagulopathy parameters D-dimer, PT, aPTT and INR values of patients who are followed up in the service and ICU of a Training and Research Hospital were Retrospectively Examined.

Whole blood parameters were studied in DXH800 (Beckman Coulter) device, ferritin and procalcitonin were studied in CobasE411 (Roche) device, CRP was studied in AU5800 (Beckman Coulter) device using turbidimetric method and coagulopathy parameters were studied in Semens Ca 7000 device using appropriate kits and materials.

Statistical Analysis

SPSS 22.0 (IBM, USA) program was used for data analysis. Kolmogorov - Simirnov and Shapiro-Wilks tests were used to examine whether the differences between the groups were normally distributed and it was observed that the data did not show a normal distribution. Therefore, nonparametric analyzes were used. Continuous variables were expressed as mean±standard deviation, and Mann-Whitney U test was used to compare parameters between groups and ($p<0.05$) was considered statistically significant. Categorical variables were expressed as frequency and percentage and compared using the Chi-square test. In addition, spearman correlation analysis was performed between the mortality rate and the analyzed parameters. G*Power 3.1.9.7 program was used to calculate the sample size. In this calculation, 110 sample size results were obtained for each group (test=t test (Wilcoxon-Mann-Whitney test (two groups)), Analysis: A priori: Compute required sample size, Tail(s)=Two, Parent distribution=Normal, Effect size d=0.5, α err prob=0.05, Power (1-β err prob)=0.95, Allocation ratio N2/N1=1).

RESULTS

115 Variant Alpha inpatients, 115 SARS-CoV-2 inpatients and 111 healthy individuals were included in this study. In this way, 3 separate groups were formed. In the sample size analysis, at least 110 sample results were determined for each group. The number of individuals included in the groups is sufficient for this result.

The mean age of the groups was determined to be 63.03±16.12 in the SARS-CoV-2 group, 59.30±15.50 in the Variant ALPHA group, and 54.72±19.90 in the control group. 51 of 115 SARS-CoV-2 patients were women, 44 of 115 Variant Alpha patients were women and 65 of 111 control group were women. The laboratory results of groups are given in table 1 as Mean±Standard Deviation.

Table 1. Laboratory findings of the groups

Parameters	**SARS-CoV-2 (n=115)**	**Variant Alpha (n=115)**	**Control (n=111)**
	Hematological parameters		
WBC (*10^9/L)	9.82±7.57	11.01±4.77	6.66±2.80
Hgb (g/dL)	11.96±2.13	11.94±1.89	12.83±1.36
PLT (*10^9/L)	232.12±99.63	221.64±96.22	242.90±102.87
Lymphocyte (*10^9/L)	1.15±0.74	0.88±0.61	1.43±0.50

Neutrophil (*10^9/L)	7.47±6.51	10.19±4.08	4.37±2.21
Monocyte (*10^9/L)	0.52±0.33	0.58±0.37	0.56±0.28
MLR	0.61±0.63	1.01±1.38	0.41±0.22
NLR	13.87±26.88	20.40±19.78	3.24±1.71
Inflammatory parameters			
PCT (mg/L)	0.54±1.73	0.72±1.79	0.09±0.10
Ferritin(ng/mL)	552.04±569.11	883.70±476.22	151.94±104.02
CRP (mg/L)	82.48±85.15	101.05±69.01	21.78±26.13
Coagulation parameters			
PT (sn)	13.29±3.51	12.19±2.12	11.39±0.72
aPTT (sn)	25.38±9.08	26.49±5.28	25.09±2.23
INR	1.17±0.33	1.06±0.19	0.98±0.07
D-dimer (µg/ml)	2.71±5.14	3.22±4.96	0.20±0.08

The laboratory results of the groups were compared. According to this; There was no difference between the Hemoglobin, Platelet, Monocyte levels of the SARS-CoV-2 group and the Variant Alpha group ($p>0.05$). On the other hand, the WBC, Neutrophil, MLR, NLR, PCT ($p = 0.037$), Ferritin, CRP, aPTT, D-dimer levels of the Variant Alpha group were higher than the levels of the SARS-CoV-2 group, and Lymphocyte ($p = 0.001$), PT, INR levels of the Variant Alpha group were lower than the levels of the SARS-CoV-2 group ($p<0.001$) as shown in table 1.

WBC, Neutrophil, MLR, NLR, PCT, CRP, Ferritin, PT, aPTT ($p=0.019$), INR and D-dimer levels of the Variant Alpha patient were higher than the levels of the control group ($p<0.001$). However, Hemoglobin ($p=0.001$) and Lymphocyte levels of the Variant Alpha patient were lower than the levels of the control group ($p<0.001$). On the other hand, there was no difference between the Platelet and Monocyte levels of the Variant Alpha patient and the control group ($p>0.05$) as shown in table 1.

WBC ($p=0.002$), Neutrophil, MLR ($p=0.009$), NLR, PCT ($p=0.001$), CRP, Ferritin, PT, aPTT, INR and D-dimer levels of the SARS-CoV-2 patient were higher than those of the control group ($p<0.001$). However, Hemoglobin ($p=0.002$) and Lymphocyte levels of the SARS-CoV-2 patient were lower than the control group ($p<0.001$). On the other hand, there

was no difference between the Platelet and Monocyte levels of the SARS-CoV-2 group and the control group (p>0.05) as shown in table 1.

44 (38.26%) of 115 Variant Alpha inpatients were hospitalized in intensive care. This rate is 38.26% in the SARS-COV-2 group. 19 (16.52%) of 115 Variant Alpha patients were ex. This rate is 24.35% in the SARS-CoV-2 group. The mortality rate observed in groups was compared using the chi-square test.

According to the chi-square analysis, there was no significant difference between the mortality rates observed in the Variant Alpha group and the SARS-CoV-2 group (p>0.05).

Spearman correlation analysis was performed to see the relationship between the Mortality rate observed in the SARS-CoV-2 and Variant Alpha groups and the parameters analyzed as shown in table 2. According to the correlation analysis; WBC, Neutrophil, MLR, NLR, PCT, Ferritin, CRP, PT, INR and D-dimer parameters were positively correlated with the mortality ratio observed in the SARS-CoV-2 and Variant Alpha groups, while the Hemoglobin, Platalet and Lymphocyte parameters were negatively correlated as shown in table 2.

Table 2. Correlation of analyzed parameters and observed mortality rate

Parameter	**Mortality rate**	
	Correlation coefficient	**p**
Hematological parameters		
WBC	0.369	<0.001
Hemoglobin	-0.175	0.008
Platalet	-0.173	0.009
Lymphocyte	-0.242	<0.001
Neutrophil	0.299	<0.001
Monocyte	0.074	>0.05
MLR	0.293	<0.001
NLR	0.325	<0.001
Inflammatory parameters		
PCT	0.339	<0.001
Ferritin	0.244	<0.001

CRP	0.270	<0.001
Coagulation parameters		
PT	0.157	0.017
Aptt	0.035	>0.05
INR	0.152	0.021
D-dimer	0.291	<0.001

DISCUSSION

Some of the SARS-CoV-2 patients undergo a period of very severe illness in the ICU, which requires treatment for ventilation and Extracorporal Membrane Oxygenation. Laboratory tests show marked changes in coagulation and immune system hyper-activation, hyperinflammation, and the resulting development of cytokine storm (8). It was determined that the severity of the disease was associated with higher Leukocyte and lower Lymphocyte count (9). It also shows that those with severe disease tend to have a higher NLR (10). In addition, an increased MLR was associated with higher mortality in these patients. MLR measured in the emergency can be useful for predicting in-hospital mortality in COVID-19 patients and It has been concluded that it may contribute to early risk classification (11).

In our study, both the SARS-CoV-2 group and the Variant Alpha group had higher WBC, Neutrophil, NLR, MLR levels and lower lymphocyte levels compared to the control group. In addition, the WBC, Neutrophil, NLR, MLR levels of the Variant Alpha group, which was called the worrying variant, were found to be higher. However, Lymphocyte levels were found to be significantly lower compared to the SARS-CoV-2 group.

Patients infected with severe SARS-CoV-2 are reported to exhibit systemic inflammatory reaction characteristics, including elevated Ferritin in the blood (12). In addition, in a study in which patients infected with SARS-CoV-2 were grouped according to the severity of the disease, the mean serum PCT levels were found to be above four times in severe patients compared to moderate patients and over eight times in critically ill patients compared to moderate patients (13). In addition, it has been stated that changes in C-reactive protein (CRP) level occured before computed tomography findings in COVID-19 patients. More importantly, CRP levels have been associated with disease development and have been shown to perform well in predicting disease severity at an early stage of COVID-19 (14).

In our study, both the SARS-CoV-2 group and the Variant Alpha group were found to be higher PCT, CRP, and Ferritin levels compared to the control group. In addition, PCT, CRP, Ferritin levels of Variant Alpha patients, which was called the worrying variant were found to be significantly higher compared to the SARS-CoV-2 patients.

Patients with coronavirus disease have high D-dimer values. In these patients, high diffuse intravascular coagulation and venous thromboembolism are described (15). In a study comparing two patient groups with severe and mild COVID-19 disease, it was stated that the patients with severe disease had abnormal coagulation parameters compared to the group with mild disease. In this study of Bao et all, it was stated that the D-dimer, PT, and INR levels were found to be higher in the patients with severe disease (16).

In our study, D-dimer, PT, aPTT, and INR levels were found to be higher in both the Variant Alpha group and the SARS-CoV-2 group compared to control group. In addition, the aPTT and D-dimer levels of the Variant Alpha group, which was called the worrying variant, were found to be higher, and PT and INR levels were found to be significantly lower compared to the SARS-CoV-2 group.

Our study suggests that the Variant Alpha group with higher levels of WBC, Neutrophil, NLR, MLR, PCT, CRP, Ferritin aPTT and D-dimer and lower Lymphocyte levels in SARS-CoV-2 patients had a more severe disease process. However, there was no significant difference between the mortality rates observed in the Variant Alpha group and the SARS-CoV-2 group. In addition, WBC, Neutrophil, MLR, NLR, PCT, Ferritin, CRP, PT, INR and D-dimer parameters were positively correlated with the mortality ratio observed in the Variant Alpha and SARS-CoV-2 groups, while Hemoglobin, Platalet and Lymphocyte parameters were negatively correlated.

It may be associated with higher PT and INR values of the SARS-CoV-2 group, no difference between the Variant Alpha group, which is thought to have a more severe disease process, and the SARS-CoV-2 group in terms of mortality rate. On the other hand, in order not to affect the analyzed parameters from the patient population, an equal number of inpatients in the intensive care unit from each 2 groups of patients were included in our study. This situation may be a source of limitation of our study. In order to see the effect of Variant Alpha on the mortality rate more clearly, a study including larger patient populations in which the rates of hospitalization and admission to intensive care in both groups are evaluated.

REFERENCES

1. Public Health England. Investigation of novel SARS-CoV-2 variant: variant of concern 202012/01, technical briefing 3. London, United Kingdom: Public Health England; 2020.

2. World Health Organization, Tracking SARS-CoV-2 variants. (Access Date: 03 October 2021). Access Address: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/

3. Frampton D, Rampling T, Cross A, Bailey H, Heaney J, Byott M, et al. Genomic characteristics and clinical effect of the emergent SARS-CoV-2 B.1.1.7 lineage in London, UK: a whole-genome sequencing and hospital-based cohort study. The Lancet Infectious Diseases 2021;21(9):1246-1256.

4. Galloway SE, Paul P, MacCannell DR, Johansson MA, Brooks JT, MacNeil A, et al. Emergence of SARS-CoV-2 B.1.1.7 Lineage —United States, December 29, 2020–January 12, 2021. MMWR Morb Mortal Wkly Rep. 2021;70(3): 95-99.

5. Vidali S, Morosetti D, Cossu E, Luisi MLE, Pancani S, Semeraro V, et al. D-dimer as an indicator of prognosis in SARS-CoV-2 infection: a systematic review. ERJ Open Research. 2020;6(2):00260-2020.

6. Singh K, Mittal S, Gollapudi S, Butzmann A, Kumar J, Ohgami RS. A meta-analysis of SARS-CoV-2 patients identifies the combinatorial significance of D-dimer, C-reactive protein, lymphocyte, and neutrophil values as a predictor of disease severity. ISLH. 2021;43(2):324-328.

7. Washington NL, Gangavarapu K, Zeller M, Bolze A, Cirulli ET, Barrett KMS, et al. Genomic epidemiology identifies emergence and rapid transmission of SARS-CoV-2 B.1.1.7 in the United States. medRxiv. 2021.02.06;21251159.

8. Skevaki C, Fragkou PC, Cheng C, Xie M, Renz H. Laboratory characteristics of patients infected with the novel SARS-CoV-2 virus. Journal of Infection. 2020;81(2):205–212.

9. Huang G, Kovalic AJ, Graber CJ. Prognostic Value of Leukocytosis and Lymphopenia for Coronaviru Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HHX, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. Journal of Infection. 2020;81(1):e6–e12.s Disease Severity. Emerg Infect Dis. 2020;26(8):1839-1841.

10. Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HHX, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. Journal of Infection. 2020;81(1):e6–e12.

11. Dávila-Collado R, Jarquín-Durán O, Solís-Vallejo A, Nguyen MA, Espinoza JL. Elevated Monocyte to Lymphocyte Ratio and Increased Mortality among Patients with Chronic Kidney Disease Hospitalized for COVID-19. J. Pers. Med. 2021;11(3):224.

12. Lin Z, Long F, Yang Y, Chen X, Xu L, Yang M. Serum ferritin as an independent risk factor for severity in COVID-19 patients. Journal of Infection. 2020;81(4):647-679.

13. Hu R, Han C, Pei S, Yin M, Chen X. Procalcitonin levels in COVID-19 patients. International Journal of Antimicrobial Agents. 2020;56(2):106051.

14. Tan C, Huang Y, Shi F, Tan K, Ma Q, MD, Chen Y, et al. C-reactive protein correlates with computed tomographic findings and predicts severe COVID-19 early. Journal of Medical Virology. 2020;32281668.

15. Al-Samkari H, Leaf RS, Dzik WH, Carlson J, Fogerty A, Waheed A, et al. COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. Blood. 2020;136(4):489–500.

16. Bao C, Tao X, Cui W, Yi B, Pan T, Young KH, et al. 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. Exp Hematol Oncol. 2020;9:16.