Araştırma Makalesi / Research Article

Folate, B12 and Iron Levels in COVID-19 Patients: Observational Case-Control Study /*COVID-19 Hastalarında Folat, B12 ve Demir Düzeyleri*: *Gözlemsel Vaka Kontrol Çalışması*

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Gönderim Tarihi | Received: 12.04.2022, Kabul Tarihi | Accepted: 21.04.2022, Yayın Tarihi | Date of Issue: 23.06.2022, DOİ: https://doi.org/10.25279/sak.1102076

Atıf | Reference: "DOĞAN, A; ANAÇ, İ; GEZER, Y; TİMUR, B. (2022). Folate, B12 and Iron Levels in COVID-19 Patients: Observational Case-Control Study. Health Academy Kastamonu (HAK), 7(Covid-19 Additional Issue) p. 91-99

Abstract

Introduction: Folic acid, B12 and Iron play an important role in the pathogenesis of viral infections. Aim: The aim of the study is to determine the blood levels of Folate, B12 and Iron and to examine their relationship with prognosis in cases followed up by the COVID-19 pandemic service. Materials and Methods: The study was planned as a retrospective observational case-control study. In a period of approximately 3 months, 140 patients were screened retrospectively, 70 of whom were COVID-19 and 70 control cases. Cases <18 years of age, followed up in the Intensive Care Unit or withdrawn from the Intensive Care Unit to the service, and patients taking drugs or supportive treatment that could affect blood Folate, B12, Iron levels were excluded from the study. $p<0.05$ was accepted as statistical significance level. Results: 48.6% (68) of the cases were male, 51.4% (72) were female. The mean age (Mean±SD) of all cases was 67.3±15.8. No significant difference was observed between the case and control groups in terms of age, gender, presence of comorbid disease, prognosis, Folate and B12 levels. However, the iron level was significantly higher in the case group. Conclusion: As a result, Folic acid and B12 supplementation is needed in the majority of COVID-19 cases. Before supplementing with iron, the iron level should be checked.

Keywords: Covid 19, B12, Folic Acid, Iron

Öz

Giriş: Folik asit, B12 ve Demir özellikle viral enfeksiyonların patogenezinde önemli rol oynamaktadır. Amaç: Bu çalışmada COVID-19 pandemi servis takibi yapılan vakalarda kanda Folat, B12 ve Demir düzeylerinin belirlenmesi ve prognoz ile ilişkilerinin irdelenmesi amaçlanmıştır. Gereç ve Yöntem: Çalışma retrospektif gözlemsel olgu-kontrol çalışması olarak planlandı. Yaklaşık 3 aylık süreçte 70 COVID-19, 70 de kontrol vakası olmak üzere 140 hasta tarandı. <18 yaş, Yoğun Bakım Ünitesi'nde takip edilmekte olan vakalar veya Yoğun Bakım Ünitesi takibi sonrasında servise çekilen vakalar ve kanda Folat, B12, Demir düzeyini etkileyecek ilaçlar veya destek tedavisi alan hastalar çalışma dışı bırakıldı. İstatistiksel anlamlılık düzeyi olarak $p<0.05$ kabul edildi. Bulgular: Vakaların %48.6'sı (68) erkek, %51.4'ü (72) kadın cinsiyet idi. Tüm vakaların yaş ortalaması (Ort±SS) 67.3±15.8 idi.

Olgu ve kontrol grupları arasında yaş, cinsiyet, komorbid hastalık varlığı, prognoz, Folat ve B12 düzeyleri açısından anlamlı fark gözlenmezken, Demir düzeyi olgu grubunda anlamlı oranda yüksekti. Sonuç: Sonuç olarak COVID-19 vakalarının büyük çoğunluğunda Folik asit ve B12 takviyesine ihtiyaç duyulmaktadır. Demir takviyesi yapmadan önce ise mutlaka demir seviyesi bakılmalıdır.

Anahtar Kelimeler: Covid 19, B12, Folik Asit, Demir

1. Introduction

Due to the COVID 19 pandemic, quarantine practices in many countries have caused malnutrition and irregular exercise (Di Renzo et. al. 2020; Muscogiuri et. al. 2020).The relationship between adequate and balanced nutrition and our immune system has been demonstrated by studies (Keusch, 2003; Bhaskaram, 2002).

Folic acid is an important subcomponent for amino acid synthesis, which is the building block of protein, and for the structure of DNA. Vitamin B12 is an important cofactor together with Folate in homocysteine synthesis. Since folate cannot be synthesized in our body, it must be taken with food. Folate deficiency occurs within a few days after insufficient intake.Folate deficiency is directly associated with cardiovascular disease, dementia and depression. In addition, megaloblastic anemia is seen due to low red blood cells through homocysteine metabolism in folic acid deficiency (Hiraoka, Kagawa, 2017; Ducker and Rabinowitz, 2017). Folic acid deficiency has also been detected in some viral infections, atypical *Mycoplasma pneumonia*, and lower respiratory tract infections, especially in the younger age group (Jacobson et. al. 1987; Strand et. al. 2007).There are studies showing that folic acid is an important factor in the immune system and antibody structure (Calder et. al. 2020; Gombart et. al. 2020).

Similarly, in a comprehensive meta-analysis examining the effect of vitamin B12 on viral infections, the immune system strengthening effect of B12 in viral infections is mentioned. It is also reported that B12 deficiency will cause memory loss and a decrease in the pain threshold (Batista et. al. 2022).

Iron plays an important role in viral replication by participating in the structure of protein structures and enzymes. It also plays a role in the host response to viral infection. Therefore, its deficiency or excess may affect the course of viral infection (Andreini et al. 2018; Williamson et. al. 2020).In this study, our aim is to determine the levels of Folate, B12 and Iron in patients requiring pandemic service admission due to COVID 19 infection and to examine their effects on the clinical course.

2. Materials and Methods

2.1. Type of Research

The study was planned as a retrospective, observational case-control study

2.2. Place and Time of Research

The study was planned at Fatsa State Hospital between 7 January and 30 March 2022.

2.3. Population, Sample and Sampling Method of Research

Seventy patients followed in the COVID 19 pandemic service were included in the case group, and 70 patients followed in non-COVID services were included in the control group. All patients included in the case group were positive for the COVID Polymerase Chain Reaction (PCR) test.Demographic characteristics, drugs used, laboratory values and prognosis of the cases were recorded. Cases who received iron, folic acid or vitamin B12 supplementation in the last 3 months, cases <18 years of age, followed in the Intensive Care Unit (ICU), or cases withdrawn from the ICU to the service were not included in the study. In addition, cases using Methotrexate, 5-Fluorouracil, Phenytoin, Valproate, Trimethoprim-sulfamethoxazole and anti-diabetic drugs that could affect folate levels were also excluded from the study. The case and control groups were compared in terms of demographic data, prognosis, and mean calculation of laboratory values. In addition, folic acid, B12 and iron levels (lower than normal, normal, higher than normal) were compared in the case group. According to the laboratory measurement range of our hospital; The normal range for folic acid level is (4.6-34.8), the normal range for B12 level is (160-800) and the normal range for iron level is (33-193).

2.4. Data Collection Tools

Hospital Information Management System (HIMS).

2.5. Data Collection

The data of the study, in which a total of 140 patients were evaluated, were obtained by scanning the Hospital Information Management System (HIMS).The data was saved to the excel program.

2.6. Ethical Considerations

Ordu University and Medical Research Ethics Committee (Decision no: 2022/116).

2.7. Statistical Analysis

The data of the study were collected retrospectively and uploaded to the IBM SPSS v.26 package program. Mean (± standard deviation) and min-max values were given for quantitative variables, and numerical (percentage) values were given for qualitative variables. "Pearson Chi-Square" and "Fisher's exact Test" were used for categorical variables. The "Kolmogorov-Smirnov" test was used for continuous variables, and it was decided that they were not normally distributed, and the "Man-Whitney U" test was used for independent quantitative data. $p<0.05$ was accepted as statistical significance level.

3. Results

48.6% (68) of the cases were male, 51.4% (72) were female. The mean age (mean±SD) of all cases was 67.3±15.8. COVID positive (+) cases and COVID negative (-) cases; Age, gender, presence of comorbid disease, admission to ICU and mortality were compared. However, no statistically significant difference was observed. The comparison of the two groups is shown in Table 1.

Table 1. Comparison of Case and Control Groups

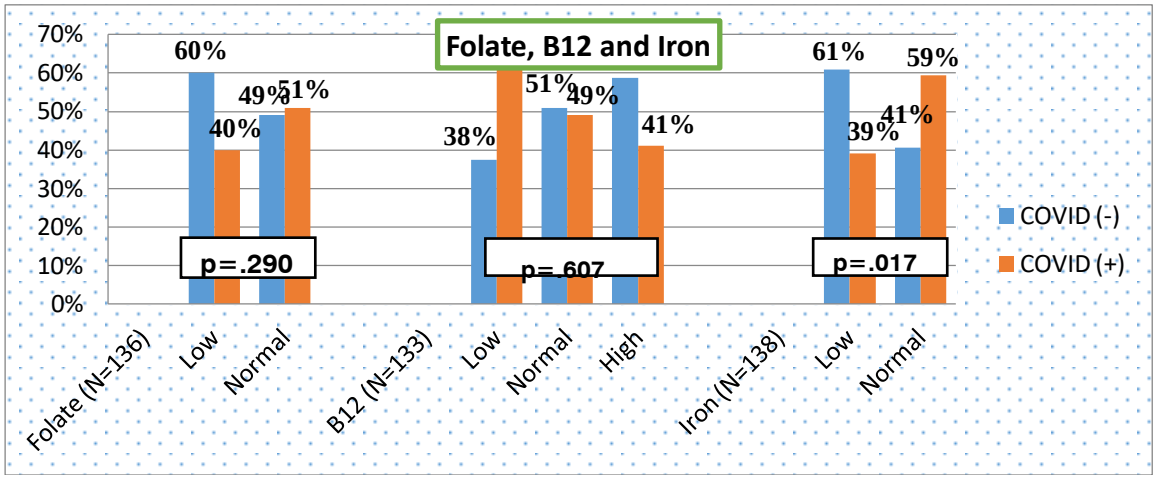
Case	COVID (-) (n=70) (%)	COVID (+) (n=70) (%)	p value
Age (Mean±SD)	66.3±15.9	68.3±16	0.299
Sex **Male** **Female**	 31 (44.3) 39 (55.7)	 37 (52.9) 33 (47.1)	0.310
Presence of comorbid disease	64 (91.4)	56 (80)	0.053
Going to ICU	4 (5.7)	8 (11.4)	0.227
Mortality	2 (2.9)	5 (7.1)	0.441

When both groups were compared by calculating the average of laboratory values; In the COVID (+) group, iron, glucose, Lactate dehydrogenesis (LDH) (U/L), Alanine aminotransferase (ALT) (U/L) and Aspartate Aminotransferase (AST) (U/L) levels were found to be significantly higher. However, the lymphocyte count was found to be significantly lower. No significant difference was observed in other values. The comparison of the laboratory values of the groups is shown in Table 2.

Table 2. Comparison of Laboratory Values of Case and Control Groups

Laboratory tests (Mean±SD)	COVID (-) (n=70)	COVID (+) (n=70)	p value
Folic acid (μg/L)	6.8±3.6	7.1±3.4	0.416
B12 (ng/L)	480.3±422.6	449.8±355.2	0.962
Iron (μg/dL)	34±25.9	49.6±31.5	**0.000**
Iron binding capacity (μg/dL)	214.4±87.7	206.4±76.3	0.737
Ferritin (μg/L)	413.7±489.6	375.8±379.6	0.795
blood white cells (WBC) (10^3/μL)	10.9±5.2	9.7±5.2	0.237
Hemoglobin(g/dL)	11.1±2.2	11.8±1.9	0.077
HCT (%)	34.6±6.2	36.7±5.6	0.065
MCV (fL)	87.1±10.2	88.4±7.4	0.624
Platelet (10^3/μL)	245.2±104.6	225.3±85.3	0.369
Neutrophil(10^3/μL)	8.4±5	7.8±3.9	0.879
Lymphocyte(10^3/μL)	1.8±1.7	1.4±3.2	**0.000**
Glucose(mg/dL)	138.9±63.9	171.1±71	**0.000**
Urea(mg/dl)	53.7±32.3	62.4±37.7	0.141
Creatinine(mg/dl)	1.3±1.1	1.2±1	0.475
ALT (U/L)	18.9±17.8	29.3±39.1	**0.004**
AST (U/L)	21±17.4	32.4±33.6	**0.002**
Gamma Glutamyl Transferase (GGT) (U/L)	104.2±115.6	96.9±107.8	0.939
Alkaline phosphatase (ALP) (U/L)	95.1±92.6	76.4±47.4	0.219
LDH(U/L)	105.1±71.4	219.8±164.5	**0.002**
C-reactive protein (CRP) (mg/dl)	124±110.3	89.5±87	0.079

According to the normal laboratory measurement range of our hospital; Based on the (4.6-34.8) range for folic acid level, (160-800) range for B12 level, and (33–193) range for iron level, reanalysis was performed in COVID 19 (+) and COVID 19 (-) groups. According to the normal range; low, normal and high values were compared in both groups. Low folate was found in 40% of COVID (+) cases, low B12 in 63%, and low iron in 39%. However, no statistically significant difference was observed between the two groups. Statistical analysis is shown in graphıc 1.



Graphic 1. Comparison of Folic Acid, B12 and Iron Levels of COVID (+) and COVID (-) Cases

In COVID (+) cases; Folic acid, B12 and iron levels were also compared according to age >50 years, comorbid status and prognosis. However, no significant difference was observed. The statistical analysis performed is shown in Table 3.

Table 3.Evaluation of Folic Acid, B12 and Iron Levels in COVID (+) Cases

Case	Folic acid low (n=12) (%)	Folic acid normal (n=54) (%)	p value
Age>50	12 (100)	44 (81.5)	0.187
Comorbidity	11 (91.7)	42 (77.8)	0.434
Going to the ICU	1 (8.3)	5 (9.3)	1.000
Mortality	1 (8.3)	2 (3.7)	0.458
	B12 low (n=5) (%)	**B12 normal/high (n=60) (%)**	
Age>50	5 (100)	10 (83.3)	1.000
Comorbidity	4 (80)	47 (78.3)	1.000
Going to the ICU	0 (0)	7 (11.7)	1.000
Mortality	0 (0)	4 (6.7)	1.000
	Iron low (n=27) (%)	**Iron normal (n=41) (%)**	
Age>50	23 (85.2	36 (87.8)	1.000
Comorbidity	21 (77.8)	33 (80.5)	0.787
Going to the ICU	4 (14.8)	4 (9.8)	0.703
Mortality	3 (11.1)	2 (4.9)	0.379

4. Discussion

In a study conducted during the COVID 19 pandemic period, 11% decreased folate level was found in patients with COVID-19. The prognosis of the cases was examined with the folate level. However, no significant relationship was observed (Meisel et. al. 2021). However, in another study, the level of folic acid was investigated according to the severity of the disease. According to mild and moderate disease severity, folic acid level was found to be lower in patients with severe clinic (Itelman et. al. 2020). Another hypothesis is that pregnant women have a protective factor that reduces the likelihood of hospitalization for SARS-CoV-2 infection tenfold compared to data obtained during the 2009 H1N1 epidemic. It has been deduced that folic acid used during pregnancy is a possible protective factor (Acosta-Elias and Espinosa-Tanguma, 2020). In our study, folic acid levels were evaluated comparatively in COVID (+) and (-) cases. Laboratory mean was found to be similar in both

groups. When folate levels were analyzed compared to the normal range (4.6-34.8), folic acid level was found to be low in 40% of COVID (+) cases. Again, in COVID (+) cases, folate level was examined according to cases over 50 years of age and comorbidity, but no significant decrease was observed. No difference was observed in terms of admission to ICU and mortality. The fact that our study was limited to 70 COVID (+) cases may have affected the folate-patient prognosis relationship.

Cellular and humoral immunity forms the basis of the defense mechanism formed against various internal and external attacks of the body. The defense mechanism is realized by various mechanisms. T helper (Th) lymphocytes carrying CD4 glycoprotein on their surface play an important role in the regulation of immunity. B vitamins are essential for cytotoxic cellular immunity and modulate T cell responses (Elmadfa and Meyer, 2019). In a hypothesis, vitamin B12 was reported as a modifiable risk factor affecting the rate of mortality and morbidity in cases of COVID 19, especially in those with advanced age and a history of Diabetes Mellitus (Wee, 2021). In another study, COVID 19 cases followed in the ICU were examined and B12 deficiency was detected in 2% of the cases (Rodriguez et. al. 2001).In our study, although low B12 was detected in 63% of the case group, no significant difference was observed between the case and control groups in terms of blood vitamin B12 levels. In addition, there was no significant difference in the prognosis of both groups. However, B12 level was found to be relatively lower than the normal range in the advanced age group and in cases with high comorbidity. B12 deficiency was 38% in the COVID (-) group, while it was 63% in the COVID (+) group. We think that this ratio will increase as the number of cases increases and a significant amount of B12 deficiency will be detected in the COVID (+) group.

Iron plays a central role in the immune response. Iron deficiency impairs Th1 cell-mediated cellular immunity. On the one hand, it is essential for many pathogens, so that its presence determines the growth and activity of these pathogens. Therefore, the distribution of iron in the body must be tightly controlled and regulated to limit the access of potentially harmful microorganisms to this trace element (Weiss et. al. 2019). Excessive iron concentrations due to excess iron intake have an inhibitory effect on macrophages, making them more susceptible to infections by certain intracellular bacteria (Ward et. al. 2011). In our study, iron deficiency was significantly higher in the COVID (-) group than in the COVID (+) group ($p=0.017$). We think that the higher iron level in COVID (+) cases may be a co-factor affecting the development of COVID in these cases. Although there was a significant difference in iron levels between the case and control groups, no significant difference was observed in terms of prognosis.

5. Conclusion

In our study, folic acid deficiency was observed in 40% and B12 deficiency in 63% of COVID (+) cases.The fact that iron deficiency is significantly higher in COVID (+) cases may be a co-factor affecting the pathogenesis of the disease. We think that B12 deficiency will be detected at a significant rate in COVID cases when a study is conducted with higher case numbers. As the number of cases increases, the relationship between vitamin deficiency and prognosis will become clear. Our study shows that COVID cases seriously need Folic acid and B12 supplements. This need increases especially in those with advanced age and comorbid diseases.

Declarations: This article was not produced from the thesis study. It was sent to the 10th Turkey EKMUD Scientific Congress to be held on 25-29 May 2022 as a verbal presentation. No kind of support has been received from anyinstitution / organization / person. The authors declared that there is no conflict of interest. Necessary permissions were obtained from Ordu University and Medical Research Ethics Committee to conduct the study (Decision no: 2022/116). All directives of the Helsinki Declaration have been followed.We would like to thank all Fatsa State Hospital Biochemistry laboratory and service staff who contributed to the study. Author contributions: İdea: AD, İA. Design: AD, İA, YG, BT. İnspection: AD, İA, YG, BT. Resources: AD, İA, YG, BT. Materials: AD, İA. Data collection and/or processing: AD, İA, YG. Analysis and/or interpretation: AD, İA, YG, BT. Literature research: AD, İA. Writing: AD, YG. Critical review: AD, İA, YG, BT

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