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DETERMINATION OF HEMOGLOBIN A1c, LIPID PROFILES, HOMOCYSTEINE, OXIDATIVE STRESS AND PHYSICAL ACTIVITY LEVELS IN DIABETIC AND/OR NON-DIABETIC COVID-19 PATIENTS

TİP II DİYABET VE/VEYA COVID-19 TANILI HASTALARDAKİ HEMOGLOBİN A1c, LİPİT PROFİLLERİ, HOMOSİSTEİN, OKSİDATİF STRES VE FİZİKSEL AKTİVİTE DÜZEYLERİNİN BELİRLENMESİ

Kadirhan Özdemir[1*], Ercan Saruhan[2], Gözde Kaya[1], Tuba Kaya Benli[3], Orhan Meral[4], Hümeyra Bozoğlan[5], Halit Demir[6], Canan Demir[7], Servet Kavak[8]

[1]Department Physiotherapy and Rehabilitation, Faculty of Health Sciences, İzmir Bakircay University, İzmir, Turkey

[2]Department of Medical Biochemistry, Faculty of Medicine, Muğla Sıtkı Koçman University, Muğla, Turkey

[3]Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Bolu Abant Izzet Baysal University, Bolu, Turkey

[4]Department of Forensic Medicine, Faculty of Medicine, İzmir Bakircay University, İzmir, Turkey

[5]Department of Endocrinology and Metabolic Diseases, İzmir Medicana International Hospital, İzmir, Turkey

[6]Department of Biochemistry, Faculty of Science, Van Yüzüncü Yıl University, Van, Turkey

[7]Health Services Vocational High School, Van Yüzüncü Yıl University, Van, Turkey

[8]Department of Biophysics, Faculty of Medicine, İzmir Bakırçay University, İzmir, Turkey

ABSTRACT

Objective: The coronavirus disease 2019 (Covid-19) pandemic results in higher morbidity and mortality rates. This study aims to compare hemoglobin A1c (HbA1c), lipid profiles, homocysteine, oxidative stress, and physical activity levels between diabetic Covid-19 patients (group I), non-diabetic Covid-19 patients (group II), and healthy controls (group III).

Method: HbA1c, lipid profiles, homocysteine, oxidative stress, oxidant, and antioxidant status were analyzed from the venous blood samples and physical activity levels were measured with International Physical Activity Questionnaire. All the parameters were compared between the groups (n=16 for each group).

Results: HbA1c and fasting blood glucose levels in group I were higher than group II and III ($p<0.001$). There were significant differences in serum levels of total cholesterol, triglyceride, high density lipoprotein cholesterol and low-density lipoprotein cholesterol between the groups ($p<0.001$). There was lower superoxide dismutase ($p<0.001$) and total antioxidant status (TAS) ($p<0.001$), and higher malondialdehyde ($p<0.001$), total oxidant status (TOS) ($p<0.001$), oxidative stress index ($p<0.001$), C-reactive protein level ($p=0.001$), and neutrophil/lymphocyte ratio ($p<0.001$) in group I and II compared with group III. Although the patients in group I and II had lower physical activity levels than group III, only the patients in group II had significantly lower physical activity levels than group III ($p=0.006$).

Conclusion: Diabetic Covid-19 patients had higher blood glucose markers than non-diabetic Covid-19 patients and healthy controls in our study. Diabetic and non-diabetic patients had higher oxidant, lower antioxidant, and higher oxidative stress levels than healthy controls. In addition, the physical activity levels of non-diabetic Covid-19 patients were lower than diabetic Covid-19 patients and healthy controls.

Key Words: Covid-19, Lipid Markers, Homocysteine, Oxidative Stress Level, Physical Activity

ÖZ

Amaç: Koronavirüs 2019 (Covid-19) pandemisi, yüksek morbidite ve mortalite oranlarına neden olmaktadır. Bu çalışma, diyabetik Covid-19 hastaları (grup I), diyabetik olmayan Covid-19 hastaları (grup II) ve sağlıklı kontroller (Grup III) arasındaki hemoglobin A1c (HbA1c), lipid profilleri, homosistein, oksidatif stres ve fiziksel aktivite düzeylerini karşılaştırmayı amaçlamaktadır.

Yöntem: Katılımcıların venöz kan örneklerinden HbA1c, lipid profilleri, homosistein, oksidatif stres, oksidan ve antioksidan seviyesi analiz edildi ve Uluslararası Fiziksel Aktivite Anketi ile fiziksel aktivite seviyeleri ölçüldü. Tüm parametreler gruplar arasında karşılaştırıldı (her grup için n=16).

Bulgular: Grup I'de HbA1c ve açlık kan şekeri düzeyleri grup II ve III'e göre yüksekti ($p<0.001$). Gruplar arasında serum total kolesterol, trigliserit, yüksek yoğunluklu lipoprotein kolesterol ve düşük yoğunluklu lipoprotein kolesterol düzeyleri arasında anlamlı fark vardı ($p<0.001$). Grup III ile karşılaştırıldığında grup I ve grup II'de daha düşük süperoksit dismutaz ($p<0.001$) ve toplam antioksidan seviyesi (TAS) ($p<0.001$), daha yüksek malondialdehit, total oksidan seviyesi (TOS) ($p<0.001$), oksidatif stres indeksi ($p<0.001$), C-reaktif protein seviyesi ($p=0.001$) ve nötrofil/lenfosit oranı ($p<0.001$) vardı. Grup I ve II'deki hastaların fiziksel aktivite düzeyleri grup III'e göre daha düşük olmasına rağmen, yalnızca grup II'deki hastaların fiziksel aktivite düzeyleri grup III'e göre anlamlı olarak daha düşüktü ($p=0.006$).

Sonuç: Çalışmamızdaki diyabetik Covid-19 hastalarının kan şekeri belirteçleri, diyabetik olmayan Covid-19 hastalarına ve sağlıklı kontrollere göre daha yüksekti. Diyabetik ve diyabetik olmayan Covid-19 hastaları sağlıklı kontrollere göre daha yüksek oksidan, daha düşük antioksidan ve daha yüksek oksidatif stres düzeyine sahipti. Ayrıca diyabetik olmayan Covid-19 hastalarının fiziksel aktivite düzeyleri diyabetik Covid-19 hastalarına ve sağlıklı kontrollere göre daha düşüktü.

Anahtar Kelimeler: Covid-19, Lipit Profilleri, Homosistein, Oksidatif Stres Seviyesi, Fiziksel Aktivite

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***Sorumlu yazar/Corresponding author:** Izmir Bakircay University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Izmir, Turkey
[1*]Email: kadirhan.ozdemir@bakircay.edu.tr, [2]Email: e.saruhan@yahoo.com, [3]Email: gozde.kaya@bakircay.edu.tr, [4]Email: tuba.kayabenli@ibu.edu.tr, [5]Email: orhan.meral@bakircay.edu.tr, [6]Email: humeyrabozoglan@gmail.com, [7]Email: halitdemir@yyu.edu.tr, [8]Email: canandemir@yyu.edu.tr, [9]Email: servet.kavak@bakircay.edu.tr

INTRODUCTION

The coronavirus disease 2019 (Covid-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) results in significantly higher morbidity and mortality rates in patients with concomitant diseases such as diabetes mellitus (DM), hypertension, coronary artery disease, cerebral infarction, and chronic bronchitis [1].

It was reported that DM is associated with a poor prognosis of Covid-19, and better glycemic control in Covid-19 patients is closely related to improved clinical outcomes [2]. International Expert Committee announced hemoglobin A1c (HbA1c) ≥ 6.5% (48 mmol/mol) as a diagnostic criterion for DM in 2009 [3].

Patients with DM typically have higher total cholesterol (TC), higher triglycerides (TG), lower high density lipoprotein cholesterol (HDL-C), and elevated amounts of low-density lipoprotein cholesterol (LDL-C) [4]. It was known that alterations in lipid profiles are related to the severity of the underlying infection [5].

Notably, the clinical condition and presence of concurrent comorbidity in Covid-19 patients serve as reasonable prognostic indications. In addition, it may be instructive to measure blood levels of coagulation factors (D-dimer and fibrinogen) and homocysteine [6]. Recently, homocysteine was suggested as a potential prognostic biomarker for the severity of Covid-19 infection [7].

Oxidative stress enhances in severe infections due to the production of free radicals, which are neutralized by antioxidants through their scavenging power, halting chain reactions, peroxide breakdown, metal chelating, and the stimulation of antioxidant enzymes [8]. During lipid peroxidation, malondialdehyde (MDA), which can be easily detected from biological structures and is a marker of peroxidative damage, is formed. MDA is an important indicator of oxidative stress; it disrupts the structures of membranes, changes their permeability, and affects functions such as ion transport and enzymatic activities [9].

Superoxide is one of the main reactive oxygen species (ROS) in oxygen metabolism. It is converted into molecular oxygen and peroxide by the enzyme superoxide dismutase (SOD). SOD, the first line of defense against ROS, is one of the most studied antioxidant metalloenzymes [10]. SOD is an essential antioxidant that the body uses to protect itself from oxidative stress. SOD is an antioxidant enzyme most found in the body that converts superoxide into other less toxic compounds [11].

The overall antioxidant and oxidation status of the body are estimated by total antioxidant status (TAS) and total oxidant status (TOS), respectively [12, 13]. Despite TAS and TOS having critical roles in the pathophysiology of Covid-19, they have rarely been investigated.

As Covid-19 spreads worldwide, people were asked to stay at home for a long time. Regular and daily living activities were decreased due to isolation and limitations. To prevent the potentially negative effects of protective lifestyle regulations due to Covid-19 and prevent restrictions from causing physical inactivity, public health professionals strongly encourage physical activity in the home environment [14].

We aimed to compare HbA1c, homocysteine, lipid profiles, oxidative stress, and physical activity levels between non-diabetic Covid-19 patients, diabetic Covid-19 patients, and healthy controls.

METHOD

This prospective controlled study was conducted in Izmir Bakircay University Cigli Training and Research Hospital. All the participants received informed consent. Ethical approval was obtained from the Ethics Committee of Izmir Bakircay University (2020-143). Participants were divided into three groups. Diabetic (type II DM) Covid-19 patients were in group I (n=16), non-diabetic Covid-19 patients were in group II (n=16), and healthy controls were in group III (n=16). According to the patient files, polymerase chain reaction (PCR) and/or computerized tomography (CT) was used to diagnose Covid-19 for the patients in group I and II. In addition, confirmatory PCR testing was performed to confirm Covid-19 diagnosis for the patients in group I and group II after hospitalization; and eliminate Covid-19 for healthy controls in group III.

The inclusion criteria were participating voluntarily in the study, being aged between 45 to 65 for all the groups, having diagnosed with type II DM and Covid-19 for group I, having diagnosed with Covid-19 for group II and no underlying comorbidities or Covid-19 for group III. The medical diagnosis of diabetes was obtained from the patient file. It was confirmed that participants in group II and group III did not have DM according to American Diabetes Association (ADA) criteria [15]. In addition, only moderate Covid-19 patients were included in this study. Patients with moderate disease had clinical signs of pneumonia but no signs of severe pneumonia, including SpO2 of 90% in room air. The exclusion criteria were being pregnant and having body mass index ≥ 40 kg/m^2 for all the groups. In addition, being an outpatient admission to the hospital and having a diagnosis of type I DM, hypertension, and heart failure were excluded from the study for patients in groups I and II.

Outcome Measures

HbA1c, lipid profiles (TC, TG, HDL-C, and LDL-C), homocysteine, D-dimer, fasting blood glucose, oxidant status (MDA and TOS) and antioxidant status (SOD and TAS), inflammatory markers (C-reactive protein (CRP), neutrophil/lymphocyte ratio (NLR)), and physical activity levels were evaluated for diabetic and non-diabetic Covid-19 patients on the first day of hospital admission following the diagnosis of Covid-19 infection. All these measurements were taken once before mid-day from the patients and healthy controls. Venous blood samples were centrifuged at 5000 × g for 10 minutes. In addition, serum samples were aliquoted and kept at -80°C until biochemical analyses.

The immunoturbidimetric assay was used in the medical biochemistry laboratory in order to determine the HbA1c levels. The parameters were studied on the Cobas Integra 800 biochemical analyzer (Roche Diagnostics, GmbH, Mannheim, Germany).

Enzyme Chemiluminescence Immunoassay (ECLIA) was used to measure homocysteine levels, and the photometric method was used to measure antioxidant parameters like SOD and MDA in biochemistry laboratory. The measurement of MDA, a lipid peroxidation product, was made by the Uchiyama and Mihara method [16]. The method is basically based on the absorbance of the pink-red color formed by the reaction of thiobarbituric acid and MDA at high temperature at 532 nm based on wavelength measurement. SOD activity was performed according to the method determined by Popov et al. [17]. Commercial kits produced by Rel Assay Diagnostics (Turkey) were used for serum TOS and TAS measurements. Erel's method was used to measure the levels of TAS and TOS in serum using automated colorimetric methods. The oxidative stress index (OSI) was calculated using the following formula: OSI (arbitrary units) = TOS (μmol H2O2 Eq/L)/TAS (μmol uric acid Eq/L) [12, 13].

The level of physical activity over the previous week was calculated by using the International Physical Activity Questionnaire-Short Form (IPAQ-SF). This questionnaire has seven questions, and the answers provide information about how much time is spent sitting, walking, doing moderate activities, and doing vigorous activities. The total score is based on the total time spent walking, doing moderate activity, and doing vigorous activity. Each activity in the questionnaire should be done for at least 10 minutes for calculation. Physical activity levels were categorized as low (<600 metabolic equivalents of task (MET)-

min/week), moderate (600-3000 MET-min/week), and high (>3000 MET-min/week). In addition, participants were defined as physically inactive when the MET value equals zero [18].

Ethical Approval

Ethical approval was obtained from the Ethics Committee of Izmir Bakircay University (2020-143).

Statistical Analysis

G*Power software 3.1 (Düsseldorf University, Germany) was used to calculate the sample size and compute the statistical power analysis. In a study including diabetic Covid-19 patients, non-diabetic Covid-19 patients, and healthy controls, the effect size of the difference between HbA1c levels was found to be f=0.476 [19]. We calculated that at least 16 participants should be included in each group in our study to obtain an effect size of at least f=0.476 for 80% power and α=0.05. The normality of data distribution was assessed with the Shapiro-Wilk test. Descriptive statistics and frequency distributions from the demographic and clinical characteristics were reported as mean, standard deviation (SD), number (n), and percentage (%). Categorical variables were analyzed using the Chi-square test for statistical significance. For normally distributed numerical variables, the statistically significant differences were determined using the one-way analysis of variance (ANOVA). The Bonferroni method adjusted p-values after the Chi-square and the one-way ANOVA analyses. The IBM SPSS Statistics software (version 14.01; SPSS, Inc., Chicago, IL, USA) was used to conduct the statistical analysis. When the p-value was less than 0.05, statistical significance was shown.

RESULTS

The demographic and clinical characteristics of the participants are shown in Table 1. Most participants were male in all the groups (Table 1). Table 1 also showed the diagnosis tools for Covid-19 in group I and II according to the patient files. In addition, confirmatory PCR test results demonstrated that all the patients in group I and II had Covid-19 infection and none of the participants had Covid-19 in group III.

Table 1. The demographic and clinical information of the participants

Variables		Group I (n=16)	Group II (n=16)	Group III (n=16)	p
Age(y) mean±SD		55.17±7.72	57.60±10.2	55.28±7.74	0.257
Gender n(%)	Male	11(68.8)	12(75.0)	12(75.0)	0.900
	Female	5(31.2)	4(25.0)	4(25.0)	
Diagnostic tool n(%)	PCR	1(6.3)	0(0)	0(0)	0.097
	CT	5(31.3)	1(6.3)	0(0)	
	PCR+CT	10(62.4)	15(93.7)	0(0)	
HbA1c levels (%) n(%)	4.7-5.6	0 (0)^{a,b}	11(68.8)^{b,c}	16(100)^{a,c}	<0.001
	5.7-6.4	6(37.5)^{b}	5(31.3)^{b}	0(0)^{a,c}	
	6.5-7.4	1(6.3)	0(0)	0(0)	
	≥7.5	9(56.3)^{a,b}	0(0)^{c}	0(0)^{c}	
FBG levels (mg/dL) n(%)	70-100	1(6.3)^{b}	5(31.3)^{b}	16(100)^{a,c}	<0.001
	100-125	1(6.3)	4(25.0)	0(0)	
	≥126	14(100)^{a,b}	7(43.8)^{b,c}	0(0)^{a,c}	
Physical activity category n (%)	Low	11(68.8)^{a}	16(100)^{b,c}	5(31.3)^{a}	0.001*
	Moderate	2(12.5)	0(0)^{b}	7(43.8)^{a}	
	High	3(18)	0(0)	4(25)	

*SD: Standard Deviation, PCR: Polymerase Chain Reaction, CT: Computerized Tomography, Hba1c: Hemoglobin A1c, Group I: Patients Diagnosed with Diabetes Mellitus and Covid-19, Group II: Patients Diagnosed with Covid-19, FBG: Fasting Blood Glucose, Group III: Healthy Controls, A: Significant Difference Compared to Group II, B: Significant Difference Compared to Group III, C: Significant Difference Compared to Group I, *p<0.05, Chi Square Test.*

The presenting symptoms of diabetic and non-diabetic Covid-19 patients are shown in Table 2. Diabetic Covid-19 patients had diabetes diagnosis for 9.75±4.48 years. While 14 out of 16 (87.5%) diabetic Covid-19 patients have been used oral antidiabetics for 9.29±4.97 years, 5 out of 16 (31.3%) of them have been used insulin for 1.69±0.48 years.

Table 2. Presenting symptoms of diabetic and non-diabetic Covid-19 patients*

Symptoms	Group I n (%)	Group II n (%)
Loss of appetite	-	1 (6.3)
Nausea	3 (18.8)	1 (6.3)
Headache	-	1 (6.3)
Sore throat	-	3 (18.8)
Joint pain	-	1 (6.3)
Muscle pain	-	4 (25.0)
Loss of sense of taste	-	1 (6.3)
Dizziness	2 (12.5)	1 (6.3)
Cough	11 (68.8)	8 (50.0)
Fever	3 (18.8)	4 (25.0)
Weakness	5 (31.3)	5 (31.3)
Loss of smell	2 (12.5)	-
Shortness of breath	6 (37.5)	4 (25.0)
Back pain	1 (6.3)	-
Phlegm	-	1 (6.3)
Diarrhea	-	1 (6.3)

*Group I: Patients Diagnosed with Diabetes Mellitus and Covid-19, Group II: Patients Diagnosed with Covid-19, *Total numbers (n) and percentages (%) of presenting symptoms are higher than the number of cases because more than a third of the patients experienced more than one symptom.*

Comparisons of the blood parameters are presented in Table 3. HbA1c and fasting blood glucose levels in group I were higher than group II and group III (p<0.001). There were no significant differences in homocysteine levels between the groups (p=0.368). There were significant differences in the levels of all the lipid profiles in group I and II compared with group III (p<0.001). Although TC and LDL-C levels in group I and group II were lower than group III (p<0.001), these lipid profile levels were in the normal reference ranges. In addition, HDL-C levels of group I and group II were also lower than healthy controls (p<0.001). TG levels of group I were above the normal reference ranges and higher than group II and group III (p<0.001). In addition, there were significant differences in group I and group II compared with group III for CRP levels (p=0.001), and NLR (p<0.001) (Table 3). Moreover, SOD, MDA, TAS, TOS levels and OSI were statistically different between all the inter group comparisons (group I vs. group II, group I vs. group III and group II vs. group III) (p<0.001) (Table 4). MDA, TOS and OSI levels were highest in group I, while SOD and TAS levels were highest in group III.

According to the physical activity levels, patients in group II had a significant difference compared to group III; however, there was no significant difference in the levels between group I and group III (Table 4). While only 31.3% of the participants were in a low physical activity category in group III, 68.6% and 100% of the patients were in the low physical activity level category in groups I and II, respectively (Table 1). In addition, 56.3% of non-diabetic Covid-19 patients, 25% of diabetic Covid-19 patients and 12.5% of healthy controls were physically inactive (p=0.003). The statistical significance of physical inactivity results was due to the difference between non-diabetic Covid-19 patients and healthy controls.

Table 3. Comparisons of the blood parameters between the groups

Data with normal reference ranges[#]	Group I Mean±SD	Group II Mean±SD	Group III Mean±SD	p
HbA1c (4.8-5.9 %)	8.43±2.85[a,b]	5.57±0.30[c]	5.48±0.34[c]	<0.001
FBG (74-109 mg/dL)	247.57±135.52[a,b]	147.79±117.57[c]	91.06±9.76[c]	<0.001
HCY (5-15 µmol/L)	8.90±1.93	10.19±3.64	9.64±1.64	0.368
TC (0-200 mg/dL)	153.94±32.92[b]	149.00±45.19[b]	210.88±31.21[a,c]	<0.001
TG (0-200 mg/dL)	238.91 ± 110.08[a,b]	139.05 ± 33.41[c]	123.88 ± 55.10[c]	<0.001
HDL-C (35-55 mg/dL)	27.64±7.72[b]	26.78±6.62[b]	57.77±15.01[a,c]	<0.001
LDL-C (0-130 mg/dL)	81.08±21.64[b]	94.41±38.60[b]	127.75±22.00[a,c]	<0.001
D-dimer (0-550 ug/L)	1190.20±1338.15[b]	1029.16±1173.94[b]	253.75±113.66[a,c]	0.031*
CRP (0-5 mg/L)	45.69±42.61[b]	54.90±48.94[b]	2.54±5.18[a,c]	0.001*
NLR (1.71-3.39 %)	4.22±2.72[b]	4.89±2.50[b]	1.72±0.49[a,c]	<0.001

*Group I: Patients Diagnosed with Diabetes Mellitus and Covid-19, Group II: Patients Diagnosed with Covid-19, Group III: Healthy Controls, HbA1c: Hemoglobin A1c, FBG: Fasting Blood Glucose, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, HCY: Homocysteine, CRP: C-Reactive Protein, NLR: Neutrophil/Lymphocyte Ratio, a: Significant difference compared to group II, b: Significant difference compared to group III, c: Significant difference compared to group I, [#]Normal reference ranges were obtained from biochemistry laboratory data of the hospital, *p<0.05, One-way ANOVA.*

Table 4. Comparisons of oxidant and antioxidant status, and physical activity levels between the groups

Variables	Group I Mean ± SD	Group II Mean ± SD	Group III Mean ± SD	p
SOD (U/ml)	1.23±0.32[a,b]	1.41±0.23[b,c]	3.78±0.25[a,c]	<0.001
MDA (mmol/L)	39.07±0.75[a,b]	29.71±5.39[b,c]	15.03±3.63[a,c]	<0.001
TAS (mmol Trolox/L)	1.56±0.25[a,b]	1.58±0.30[b,c]	3.43±0.14[a,c]	<0.001
TOS (µmol H_2O_2/L)	5.27±0.05[a,b]	5.05±0.2[b,c]	2.25±0.2[a,c]	<0.001
OSI (Arbitrary unit)	3.38±0.52[a,b]	3.19±0.61[b,c]	0.66±0.01[a,c]	<0.001
PA (MET)	1117.48±1888.91	74.13±134.07[b]	2310.72±2591.48[a]	0.006*

*Group I: Patients diagnosed with Diabetes Mellitus and Covid-19, Group II: Patients diagnosed with Covid-19, Group III: Healthy Controls, SOD: Superoxide Dismutase, TAS: Total Antioxidant Status, TOS: Total Oxidant Status, MDA: Malondialdehyde, OSI: Oxidative Stress Index, PA: Physical Activity, a: Significant difference compared to group II, b: Significant difference compared to group III, c: Significant difference compared to group I, *p<0.05, One-way ANOVA.*

DISCUSSION

To the best of our knowledge, this is the first study to compare HbA1c, lipid profiles, homocysteine, oxidative stress, and physical activity levels between diabetic and non-diabetic Covid-19 patients, and healthy controls. This study demonstrates that diabetic Covid-19 patients had higher HbA1c and fasting blood glucose levels than non-diabetic Covid-19 patients and healthy controls. The levels of TC, HDL-C and LDL-C in diabetic and non-diabetic Covid-19 patients were different from healthy controls. The HDL-C levels were under the normal reference ranges for diabetic and non-diabetic Covid-19 patients. In addition, TG levels were above the normal reference ranges for diabetic Covid-19 patients. Although there were no differences in homocysteine levels between the groups, D-dimer levels of diabetic and non-diabetic Covid-19 patients were higher than healthy controls. Both diabetic and non-diabetic Covid-19 patients had higher CRP levels and NLR than healthy controls. In addition, SOD, MDA, TAS, TOS levels, and OSI were different between all the groups. Diabetic Covid-19 patients had the lowest SOD and TAS levels, and the highest MDA, TOS and OSI levels. According to the physical activity levels, non-diabetic Covid-19 patients were less physically active than diabetic Covid-19 patients and healthy controls.

We would like to emphasize which parameters should be under-controlled for a better Covid-19 prognosis by determining the initial HbA1c, lipid profiles, homocysteine, oxidative stress parameters, and physical activity levels of diabetic and non-diabetic Covid-19 patients compared with healthy controls.

The severity of the infection is inversely proportional to the severity of the changes in the levels of lipid profiles. A recent study found that severe cases of Covid-19 were associated with lipid metabolic problems [5]. According to the results of our study only HDL-C and TG levels were out of the normal reference ranges for diabetic Covid-19 patients. In addition, HDL-C levels were lower than normal ranges in both diabetic and non-diabetic Covid-19 patients. These results may show that our study is partially compatible with the literature, because all the patients were diagnosed with moderate Covid-19. In addition, it was known that oral anti-diabetics influence lipid profiles in patients diagnosed with DM [20]. Therefore, the fact that diabetic Covid-19 patients had normal mean TC (153.94 ± 32,92 mg/dL) and LDL-C (81.08 ± 21.64 mg/dL) levels may be clarified that 87.5% of the patients were under anti-diabetic treatment. Interestingly, TC levels were higher in healthy controls compared to diabetic and non-diabetic Covid-19 patients. The reason why higher TC levels (210.88±31.21 mg/dL) of healthy controls could be explained the creation of groups with convenience sampling due to the nature of the study design. In addition, mean TC levels (210.88±31.21 mg/dL) of healthy controls were borderline high (200 to 239 mg/dL) which means slightly higher than normal reference ranges (0 to 200 mg/dL).

According to Ponti et al., homocysteine, a crucial predictor of cardiovascular disorders, is also a crucial and predictive factor in Covid-19 [7]. In addition, it is also possible to interpret a high D-dimer level at admission as a sign of intravascular coagulation, thrombotic illness, or enhanced fibrinolysis [21]. Although homocysteine levels of all groups were in the normal reference ranges, D-dimer levels of both diabetic and non-diabetic Covid-19 patients were out of the reference ranges and higher than healthy controls in our study. The fact that homocysteine levels remained within normal limits, unlike D-dimer, can be explained that both diabetic and non-diabetic Covid-19 patients did not have severe disease in our study.

There are contradictory results about the interaction between hyperglycemia and clinical outcomes of diabetic Covid-19 patients in the literature. Poorer glycemic control has been linked to worse health outcomes in type II diabetic Covid-19 patients [22]. However, hyperglycemia was not proven to be linked to clinical outcomes in diabetic Covid-19 patients [23]. In our study, diabetic Covid-19 patients had higher HbA1c levels than non-diabetic Covid-19 patients and healthy adults. However, the presenting symptoms of diabetic and non-diabetic Covid-19 patients were similar in our study. The main reason for this similarity may be that all the patients had moderate severity of Covid-19.

Patients unable to maintain normal blood glucose levels may also experience more severe inflammation. Because there was a direct connection between hyperglycemia and inflammation, liver damage and coagulation abnormalities; hyperglycemia could potentially contribute to Covid-19 patients being more severely ill [24]. Moreover, Covid-19 disease is characterized by a fulminant hypercytokinemia called a cytokine storm. It was known that, in the early stages of a systemic inflammatory response, elevated blood sugar levels and increases in the levels of certain cytokines are observed. In critical cases of these conditions, the pro-inflammatory phase is characterized by metabolic stress, which leads to glycogen breakdown, the synthesis of adrenocorticotrophic and glucagon hormones, and insulin resistance, all of which cause an increase in blood glucose [25]. In our study, 25% of the non-diabetic Covid-19 patients' the fasting blood glucose levels were between 100 - 125 mg/dL and 43.8% of them were 126 md/dL or higher, which may be a result of the inflammatory process created by Covid-19.

The information about the link of hyperinflammatory condition to diabetes has been gathered from research on type II diabetic patients. Because persistent, low-grade inflammation has long been linked to insulin resistance [26]. As anticipated, diabetic Covid-19 patients had higher levels of hyperglycemia than non-diabetic Covid-19 patients in our study. However, according to CRP and NLR levels, the inflammatory responses of diabetic and non-diabetic Covid-19 patients were similar in our study. The main reason may be that approximately 70% of non-diabetic Covid-19 patients' fasting blood glucose levels were higher than upper limits of reference ranges.

Zhang et al. recommended that clinicians pay increased attention to the blood glucose level of diabetic and non-diabetic Covid-19 patients as it may indicate poor prognosis. They also noted that blood glucose status should be critical in establishing an effective public health plan to alleviate the poor Covid-19 prognosis [22]. Recently, it was revealed that insulin infusion might be an efficient technique for meeting glycemic objectives and improving the clinical outcome of Covid-19 [27]. Sardu et al. mainly reported that tight glycemic control had a protective effect on the outcome of patients with hyperglycemia with Covid-19 infection. They also found that the reduction in glucose levels achieved by insulin infusion in Covid-19 patients was associated with better outcomes [27]. Therefore, tight control of glycemia may be required to improve the outcomes of diabetic and non-diabetic Covid-19 patients.

Oxidative stress has adverse effects on glucose uptake and insulin secretion. Additionally, increased oxidative stress is a condition that commonly contributes to the development and progression of diabetes and its complications [28]. It was known that oxidative stress impairs the ability of muscle and fat cells to absorb glucose and decreases the insulin secretion from the pancreatic beta cells [29]. For this reason, oxidative stress may be the reason why not only diabetic Covid-19 patients, but also non-diabetic Covid-19 patients had hyperglycemia in our sample. Moreover, hyperglycemia can cause oxidative stress and elevation of proinflammatory factors, ultimately leading to vascular dysfunction [30]. Few experimental data have been reported on measuring oxidative stress in patients with Covid-19. Research groups have recently generally focused on retrospective data analysis, clinical features of the disease, or review articles [31]. According to the results of our study, both diabetic and non-diabetic Covid-19 patients exhibited high oxidant status and inflammatory indicators, as well as low antioxidant status, compared to the control group.

Aging, cancer, inflammation, allergies, and some systemic or localized diseases disrupt the balance between oxidant and antioxidant capacity. When the antioxidant status is suppressed by oxidative stress, mitochondrial damage, metabolic changes such as apoptosis, disruption of cellular repair mechanisms, vascular endothelial changes, expression of inflammatory cytokines, and disease progression may occur [32]. Previous research conducted by Muhammad et al. [33], and Kumar et al. [34] demonstrates that patients with Covid-19 had lower levels of SOD. In addition, comorbidities, including DM and hypertension, put the body under oxidative stress. Infection with Covid-19 amplifies this stress, leading to a decrease in SOD and a subsequent oxidative system overload [34]. Previous research has revealed that the etiology of Covid-19 is significantly influenced by the imbalance between the oxidant and antioxidant systems [31]. However, limited studies focus on the direct role of oxidative stress and its biomarkers in patients infected with Covid-19. Muhammed et al. [33] showed that oxidant status increased, and antioxidant status decreased in Covid-19 patients. In our study, SOD, MDA, TAS, TOS levels and OSI were significantly different in diabetic and non-diabetic Covid-19 patients compared with healthy controls. Moreover, the levels of SOD, TAS, MDA and TOS, and OSI in diabetic Covid-19 patients were more significant than non-diabetic Covid-19 patients. These results demonstrated that oxidative stress increased, and the oxidant/antioxidant balance impaired in diabetic and non-diabetic Covid-19 patients. However, diabetic Covid-19 patients were more adversely affected by oxidative stress and oxidant/antioxidant balance compared with non-diabetic-Covid-19 patients. Therefore, measuring the oxidative stress and oxidant/antioxidant balance may be much more essential to determine the progression of Covid-19 in diabetic Covid-19 patients.

Physical activity has many health benefits, including weight loss, CVD prevention, better immune system function, and resistance to viral infections. The immune system adapts by improving its function depending on the exercise type and intensity [35]. Moderate exercise intensity improves immune system activity, promoting immune system adaptations. In addition, moderate physical activity is associated with a lower risk of death from respiratory disorders like Covid-19 [35]. Furthermore, regular physical exercise lowers inflammatory responses and protects tissues from the damaging effects of oxidative stress, both of which are associated with an increased risk of developing DM [36].

Physical activity is also an indispensable aspect of the DM prevention plan. Increased moderate physical activity can potentially improve the health of patients with DM, insulin resistance, and pre-DM [37]. A meta-analysis of 14 trials found that exercise significantly decreased the HbA1c levels of patients with type II DM [38]. In our study, HbA1c levels of diabetic Covid-19 patients exceeded the reference ranges. In addition, both diabetic and non-diabetic Covid-19 patients had hyperglycemia. Thus, regular physical exercise recommendations may be beneficial for regulating blood glucose in Covid-19 patients.

In the last decade, sedentary behavior has emerged as a potential risk factor for many chronic conditions and mortality raising concern during home confinement [39]. In addition to staying active throughout the day, it is recommended that at least three times per week and twenty minutes of intense physical activity be performed at a heart rate reserve of between 60 and 89 percent and a maximum of between 70 and 84 percent of the one-repetition maximum [40]. At least 5400-7900 steps per day, or about 4-6 km, is needed to meet the suggested target of moderate- and vigorous-intensity physical activity, equating to the requirement of at least 500-1000 MET minutes per week [40]. Although physical activity levels of diabetic Covid-19 patients (1117.48 ± 1888.91 METs) were similar to healthy adults (2310.72 ± 2591.48 METs), non-diabetic Covid-19 patients' levels (74.13 ± 134.07 METs) were different in our study. These results can be interpreted that Covid-19 patients, who were previously diagnosed with DM, attached more importance to physical activity in terms of their general health.

Hamer et al. reported that after adjusting for factors such as age, sex, weight, smoking, and alcohol consumption, physical inactivity is still associated with a greater risk of Covid-19 hospitalization [41]. Several other factors are working together to cause this association; however, exercise could positively impact the immune system [42]. Recent findings from a survey conducted during the Covid-19 pandemic indicated that the prevalence of physical inactivity was 57.3% among the general population and 57.7% among people at risk for developing type II DM [43]. As a striking result of our study, more than half of the non-diabetic Covid-19 patients and one-quarter of diabetic Covid-19 patients were physically inactive, and the remaining were at a low physical activity level. In addition, while none of the non-diabetic Covid-19 patients were in the moderate and high physical activity category, only 12.5% and 18% of the diabetic Covid-19 patients were in those categories, respectively. Our results supported the recent literature that physical inactivity was common because of the effect of the Covid-19 pandemic. For this reason, we recommend that people should be encouraged to do physical activity or exercise.

Limitations

First, only patients diagnosed with moderate Covid-19 were included in this study. Further studies could investigate the levels of HbA1c, lipid profiles, homocysteine, oxidative stress parameters, and physical activity levels in different stages of diabetic and/or non-diabetic Covid-19 patients. Second, we included only type II diabetic Covid-19 patients in this study. Type I diabetic Covid-19 patients should be included in future studies. Lastly, we only evaluated the baseline levels of the parameters. Therefore, the data did not reveal any information about potential changes in Covid-19 patients while they were hospitalized.

CONCLUSION

As a consequence, both diabetic and non-diabetic Covid-19 patients had higher oxidative stress level and impaired oxidant/antioxidant status than healthy controls. In addition, higher blood glucose marker levels, higher oxidant and lower antioxidant activities was seen in diabetic Covid-19 patients than non-diabetic Covid-19 patients. There were no significant differences in homocysteine levels between diabetic and non-diabetic Covid-19 patients and healthy controls. In addition, TC and LDL-C levels in diabetic and non-diabetic Covid-19 patients were lower than healthy controls; however, these lipid profile levels were in the normal reference ranges. Moreover, physical activity levels of non-diabetic Covid-19 patients were lower than diabetic Covid-19 patients and healthy controls.

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