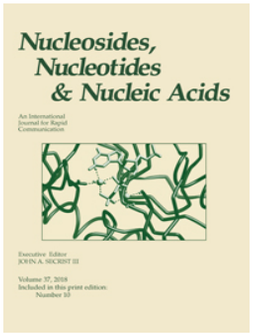

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The effect of DNA repair gene variants on COVID-19 disease: susceptibility, severity, and clinical course

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ABSTRACT

Oxidative stress (OS), which leads to DNA damage, plays a role in the pathogenesis of Coronavirus disease 2019 (COVID-19). We aimed to evaluate the role of DNA repair gene variants [X-ray repair cross complementing 4 (*XRCC4*) rs28360071, rs6869366, and X-ray cross-complementary gene 1 (*XRCC1)* rs25487] in susceptibility to COVID-19 in a Turkish population. We also evaluated its effect on the clinical course of the disease. A total of 300 subjects, including 200 COVID-19 patients and 100 healthy controls, were included in this study. These variants were genotyped using polymerase chain reaction (PCR) and/or PCR-restriction fragment length polymorphism (RFLP) methods. The patients were divided into three groups: those with a mild or severe infection; those who died or lived at the 28-day follow-up; those who required inpatient treatment or intensive care. There were 87 women (43.5%) and 113 men (56.5%) in the patient group. Hypertension was the most common comorbidity (26%). In the patient group, *XRCC4* rs6869366 G/G genotype and G allele frequency were increased compared to controls, while *XRCC4* rs6869366 G/T and T/T genotype frequencies were found to be higher in controls compared to patients. *For XRCC1* rs25487, the A/A and A/G genotypes were significantly associated with COVID-19 disease. All of the patients hospitalized in the intensive care unit had the *XRCC4* rs6869366 G/G genotype. In this study, we evaluated for the first time the impact of DNA repair gene variants on COVID-19 susceptibility. Results suggested that *XRCC4* rs6869366 and *XRCC1* rs25487 were associated with COVID-19 suspectibility and clinical course.

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1. Introduction

The Coronavirus disease 2019 (COVID-19) pandemic is an important global health and economic problem, causing high mortality and disability worldwide.[1] Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a deadly pathogen, causes the COVID-19 disease that has emerged in recent years.[2] According to the symptoms of COVID-19, cases can be mild, moderate, severe, or critical, but asymptomatic cases that can cause the virus to spread have also been reported.[3] COVID-19 severity and mortality are associated with factors such as age, gender, and comorbidities.[4] However, it has not been fully defined what determines the severity of the disease-related comorbidities There is evidence to suggest that oxidative stress (OS) and defense against reactive oxygen species (ROS) are important in the pathogenesis of COVID-19.[5] In the organism, OS results from an imbalance between antioxidant capacity and ROS in the cell.[6] ROS affects biochemical pathways in the cell, and DNA helix breaks, lipid peroxidation, and protein modification and degradation occur.[7]

The major DNA damages are single-strand breaks (SSBs) and double-strand breaks (DSBs). DSBs are the most harmful form, as they can lead to chromosomal breakage or rearrangement. Defects in the repair of DSBs can cause many pathological events, including genomic instability, cell death, and carcinogenesis.[8] It has been predicted that DNA repair defects can cause DNA damage, genomic instability, and cell cycle deregulation during the replication of viruses in host cells.[9] Single nucleotide polymorphisms (SNPs) affect protein function and gene expression, thereby forming the basis of susceptibility to disease. Variants in DNA repair genes are thought to modulate DNA repair capacity and may be associated with diseases. The protein encoded by the X-ray repair cross complementing 4 (*XRCC4*) gene is involved in the repair of DNA DSBs and functions together with DNA ligase IV and DNA-dependent protein kinase. XRCC4 is involved in the non-homologous end-joining (NHEJ) pathway, which is the key method in higher eukaryotes for DSBs.[10] *XRCC4* rs6869366 is located in the promoter region of the gene, while *XRCC4* rs28360071 is located in the intronic region. [11] *XRCC4* genetic variants have been shown to play a potential role in HIV-1 infection risk.[12] Another gene that acts as a scaffolding protein for base excision repair (BER) in the DNA repair pathway is X-ray cross-complementary gene 1 (*XRCC1*).[13] In preclinical studies, it was determined that XRCC1 deficiency causes the delay of DNA SSBs recombination, the induction of mutations, and increased sister chromatid formation, which is an indicator of genomic instability.[14]

XRCC1 Arg399Gln (rs25487) variant located in exon 10 can alter protein function and decrease DNA repair kinetics. Studies have suggested that the rs 25487 A/A genotype has a 3 to 4 fold reduced DNA repair capacity.[15]

Based on the relationship between OS and DNA damage, we aimed to evaluate the role of DNA repair gene variants (*XRCC4* rs28360071, rs6869366, and *XRCC1* rs25487) in susceptibility to COVID-19 in a Turkish population. We also evaluated its effect on the clinical course of the disease.

2. Materials and methods

2.1. Study population

The study included 200 COVID-19 patients who applied to the Istanbul University, Faculty of Medicine, Infection Clinics between April 2020 and June 2020. The COVID-19 diagnosis was confirmed by computed tomography (CT) and the positivity of the polymerase chain reaction (PCR). The patient's demographic information, such as gender, age, symptoms, comorbidities, laboratory findings, clinical findings, and physical examination findings, were recorded. The control group consisted of 100 healthy people with negative COVID-19 tests and no chronic diseases. We explained the study details fully, and all the participants submitted written informed consent before enrollment in the study. The study was conducted according to the Helsinki Declaration and approved by the Clinical Studies Ethics Committee (21/05/2020-84539). We divided the patient group into three different groups according to their clinical status.

2.2. Mild and severe infection groups

We divided the patient group into two groups: mild and severe infection groups. The patient criteria included in the "severe infection" group are as follows:

- A respiratory rate of more than 30 breaths per minute
- Lactate concentrations greater than 2 mmol/L, nasal oxygen requirements greater than 5 L/min, shortness of breath, or ambient oxygen saturation greater than 90%
- A heart rate of more than 100 beats per minute, a drop in blood pressure, or if the systolic blood pressure is 40 mm Hg lower than the normal systolic blood pressure
- Any liver, brain (such as confusion), blood (such as thrombocytopenia), or kidney dysfunction
- Cutaneous symptoms such as peripheral coldness and cutis marmorata, as well as sepsis or septic shock
- Any pneumonia, severe or mild, with multiple ground glass opacities and/or bilateral infiltration.
- Requirement for broad-spectrum antibacterial and/or anti-cytokine therapy

2.3. Dead and living groups in a 28-day follow-up

During the 28-day follow-up period, the patients were divided into two subgroups according to whether they died or survived.

2.4. Patients in intensive care and on the wards

The patients were divided into two subgroups based on their needs during the 28-day follow-up period, either for intensive care or inpatient treatment.

2.5. Genotyping

All patients and controls submitted two mL of venous blood. DNA was extracted from all the samples with the salting-out method. *XRCC4* rs28360071, rs6869366, and *XRCC1* rs25487 variants were genotyped using the polymerase chain reaction (PCR) and/or PCR-restriction fragment length polymorphism (RFLP) method described previously.[16] A representative gel image of PCR products checked in gel electrophoresis is given in Figure 1.

2.6. Statistical analysis

IBM SPSS Statistics for Windows 21.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. After assessing the continuous variables' normality, the descriptive statistics were expressed as the mean, standard deviation, median, maximum, and minimum, while frequency and percentage were used to express the nominal variables. For comparing the discrete variables, the Pearson chi-square test or Fisher exact test was used, and in the pairwise comparisons, Bonferroni correction was used to determine which group or groups showed statistically significant results. In order to determine the association between different variants of the genes and the study parameters, multivariate binary logistic regression analyses were performed. The results were adjusted for gender and age. The odds ratio (OR) and 95% confidence interval (CI) were used to express the association of the gene variants with the study parameters. Hardy-Weinberg equilibrium (HWE) was calculated with. Pearson's chi-squared test. Statistical significance was accepted as $p \leq 0.05$ in all of the analyses.

3. Results

In this case-control study, *XRCC4* rs28360071, rs6869366, and *XRCC1* rs25487 variants were examined in 200 COVID-19 patients and 100 healthy

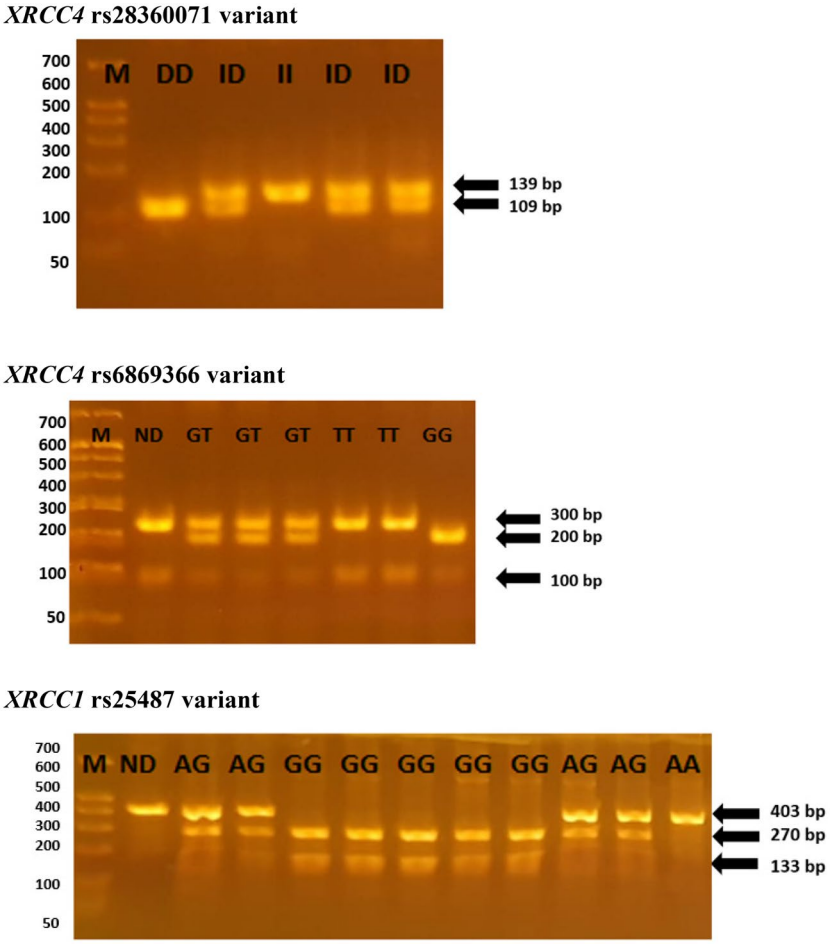

Figure 1. Agarose gel electrophoresis showing genotypes of *XRCC4* rs28360071, *XRCC4* rs6869366, and *XRCC1* rs25487 variants.

controls. There were 87 women (43.5%) and 113 men (56.5%) in the patient group. The ages of the patients ranged from 19 to 92, and the mean age was 49 years. The most common comorbidity was hypertension in 54 patients (26%). The mortality rate at 28 days of follow-up was 4.5%. Baseline demographic and clinical characteristics of the participants are shown in Table 1.

3.1. XRCC4 *rs28360071*

The D/D, I/D, and I/I genotype frequencies were 18.5% versus 28%, 50% versus 43%, and 31.5% versus 29% in patients and the control group, respectively. There was no statistically significant difference in genotype and allele distribution of the *XRCC4* rs28360071 between patients and controls ($p > 0.05$) (Table 2). There was no deviation from HWE.

Table 1. Clinical and demographic characteristics of the patients.

	COVID-19 patients n:200 (%)	Median
Age (years)		49 (19–92)
Gender	87/113 (43.5/56.5)	
Female/male		
Comorbidities		
Hypertension	54 (26)	(ARB: 19, ACE: 14)
Diabetes mellitus	31 (15.5)	
COPD	22 (11.6)	
CAD	14 (7.0)	
CHF	4 (28)	
Solid malignancy	22 (11.2)	
Hematological malignancy	5 (2.5)	
Clinical findings		
Severe/mild	94/106 (47%/53%)	
Cough	125 (62.5)	2 (0–20)
Fever	107 (53.5)	1 (0–14)
Myalgia	104 (52)	1 (0–15)
Dyspnea	62 (31)	1 (0–15)
Nausea, vomiting	23 (11.5)	1 (0–15)
Diarrhea	16 (8.1)	
Anosmia	7 (3.5)	
Sputum	1 (0.4)	
Initial examination		
Fever	94/106 (47/53)	36.7 (36–40)
spO_2		97 (80–100)
Systolic blood pressure		128 (90–200)
Diastolic blood pressure		75 (50–100)
Heart rate/min		93 (60–160)
Respiratory rate/min		16 (12–40)
pH		7.41 (7–8)
pO_2		63 (35–86)
pCO_2		38 (23–58)
HCO_3		24 (15–30)
Lactate		1.40 (1–5)
Labotory findings		
Hemoglobin (g/dL)		13.1 (6.3–16.8)
Leukocyte (μL)		7115 (220–28,300)
Thrombocyte (10^3/μL)		237 (66–576)
Lymphocyte (μL)		1340 (290–4500)
Lymphocyte (< 800 μL)	42 (21)	
Eosinophil		30 (10–2780)
Urea (mg/dL)		14 (5-107)
Creatinine (mg/dL)		0.8 (0.4–6)
Na		139 (113-172)
K		4.4 (3–6)
Glucose		108 (68–496)
AST		22 (10–409)
ALT		22 (2–493)
GGT		22 (5–744)
ALP		73 (33–400)
LDH (IU/L)		205 (78-731)
Total protein (g/L)		7.4 (5–9)
Albumin (g/L)		4.0 (2–5)
C-reactive protein (mg/dL)		20 (1-363)
Procalcitonin		0.06 (0.20–50.0)
Ferritin		179 (6-6656)
D-Dimer		620 (190-20,000)
ProBNP		58 (5–35,000)
Troponin		4 (3-848)
Fibrinogen		437 (204-1053)
INR		0.9 (0.8-3.8)
APTT		28 (21–53)
Treatment regimen		
Favipravir	61 (30.5)	
Tocilizumab	11 (5.5)	
28-day mortality	9 (4.5)	
Intesive care	16 (8)	

ARB: Angiotensin II receptor blocker, ACE: angiotensin-converting enzyme, COPD: chronic obstructive pulmonary disease, CAD: coronary artery disease, CHF: congestive heart failure, sPO_2: capillary oxygen saturation, pO_2: partial pressure of oxygen, pCO_2: partial pressure of carbon dioxide, HCO_3: bicarbonate, Na: natrium, K: potassium, AST: aspartate aminotransferase, ALT: alanine transaminase, GGT: gamma-glutamyl transferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, T. Protein: total protein, ProBNP: pro brain natriuretic peptide, INR: international normalized ratio, aPTT: activated partial thromboplastin time.

Table 2. Genotype and allele distribution of variants between the patients and controls.

XRCC4 rs28360071					
Genotypes	Patients n:200 (%)	Controls n:100 (%)	OR Exp (B)	95% CI	p
D/D	37 (18.5)	28 (28)	0.616*	0.318–1.193*	0.150*
I/D	100 (50)	43 (43)	1.094*	0.619–1.934*	0.757*
I/I	63 (31.5)	29 (29)	1.126[&]	0.666–1.903[&]	0.692[&]
Alleles					
D	174 (43.5)	99 (49.5)	0.785&	0.559–1.104&	0.166&
I	226 (56.5)	101 (50.5)			
HWE	p = 0.885	p = 0.164			
XRCC4 rs6869366					
Genotypes	Patients n:200 (%)	Controls n:100 (%)	OR Exp (B)	95% CI	p
G/G	155 (77.5)	26 (26)	55.996*	18.417–170.250*	**0.001***
G/T	41 (20.5)	36 (36)	10.642*	3.454–32.790*	**0.001***
T/T	4 (2)	38 (38)	0.033[&]	0.011–0.097[&]	**0.001**[&]
Alleles					
G	351 (87.75)	88 (44)	0.110&	0.073–0.165&	**0.001**[&]
T	49 (12.25)	112 (66)			
HWE	p = 0.008	p = 0.506			
XRCC1 rs25487					
Genotypes	Patients n:200 (%)	Controls n:100 (%)	OR Exp (B)	95% CI	p
A/A	78 (39)	34 (34)	1.903*	1.013–3.576*	**0.046***
A/G	87 (43.5)	36 (36)	2.184*	1.163–4.102*	**0.015***
G/G	35 (17.5)	30 (30)	0.482[&]	0.276–0.842[&]	**0.012**[&]
Alleles					
A	243 (60.75)	104 (52)	0.700[&]	0.497–0.986[&]	**0.044**[&]
G	157 (39.25)	96 (48)			
HWE	**p = 0.005**	p = 0.235			

XRCC4: X-ray repair cross-complementing protein 4, XRCC1: X-ray repair cross-complementing group 1, HWE: Hardy-Weinberg equilibrium *: OR (95% CI) was adjusted for age and sex, [&]Fisher exact test. The results that are statistically significant are shown in boldface.

3.2. XRCC4 *rs6869366*

There was a significant difference in *XRCC4* rs6869366 genotype and allele distribution between the patients and control subjects. For *XRCC4* rs6869366, G/G genotype and G allele frequency were increased compared to controls ($p = 0.001$, OR: 55.996, 95%Cl: 0.559-1.104; $p = 0.001$, OR: 0.110, 95%Cl: 0.073–0.165, respectively). *XRCC4* rs6869366 G/T and T/T genotypes increased in controls compared to patients ($p = 0.001$, OR: 10.642, 95%Cl: 3.454–32.790; $p = 0.001$, OR: 0.033, 95%Cl: 0.011–0.097, respectively) (Table 2). There was no deviation from HWE.

3.3. XRCC1 *rs25487*

The *XRCC1* rs25487 genotype and allele distribution were statistically different between patients and the control group. *XRCC1* rs25487 A/A and A/G genotypes were found to be higher in patients compared to

controls ($p = 0.046$, OR: 1.903, 95%Cl: 1.013-3.576; $p = 0.015$, OR: 2.184, 95%Cl: 1.163-4.102, respectively). *XRCC1* rs25487 G/G genotype increased in the control group compared to controls ($p = 0.012$, OR: 0.482, 95%Cl: 0.276-0.842). Also, the *XRCC1* rs25487 A allele was more common in the patients compared to the controls ($p = 0.044$, OR: 0.700, 95%Cl: 0.497-0.986) (Table 2). There was a deviation from HWE in the patient group ($p = 0.005$).

3.4. Mild and severe infection groups

We then compared the genotype distribution of these variants between severe and mild cases. There were 94 (47%) people in the severe infection group and 106 (53%) in the mild infection group. The *XRCC4* rs28360071, rs6869366, and *XRCC1* rs25487 genotype distributions were not different between the severe infection and mild infection groups ($p > 0.05$). Results are presented in Table 3.

3.5. Dead and living groups in a 28-day follow-up

We evaluated the genotype distributions between those who died and those who survived the 28-day follow-up. There were 9 (4.5%) people in the death group and 191 (95.5%) in the survival group. There was no significant difference in genotype distribution of *XRCC4* rs28360071, rs6869366, and *XRCC1* rs25487 between these patients after a 28-day follow-up ($p > 0.05$). Results are shown in Table 4.

Table 3. Genotype distribution of variants between the severe infection and mild infection groups.

	Severe group n = 94 (%)	Mild group n = 106 (%)	OR Exp (B)	95% CI	p
XRCC4 rs28360071 Genotypes					
D/D	17 (18.1)	20 (18.9)	1.219*	0.475–3.132*	0.680*
I/D	45 (47.9)	55 (51.9)	1.157*	0.559–2.397*	0.694*
I/I	32 (34.0)	31 (29.2)	1.249[&]	0.687–2.270[&]	0.542[&]
XRCC4 rs6869366 Genotypes					
G/G	76 (80.9)	79 (74.5)	2.628*	0.233–29.612*	0.434*
G/T	15 (16.0)	27 (24.5)	3.678*	0.300–45.102*	0.309*
T/T	3 (3.1)	1 (1.0)	3.462[&]	0.354–33.860[&]	0.344[&]
XRCC1 rs25487 Genotypes					
A/A	37 (39.4)	41 (38.7)	1.404*	0.568–3.472*	0.462*
A/G	39 (41.5)	48 (45.3)	1.962*	0.784–4.908*	0.150*
G/G	18 (19.1)	17 (16.0)	1.240[&]	0.597–2.573[&]	0.581[&]

XRCC4: X-ray repair cross-complementing protein 4, XRCC1: X-ray repair cross-complementing group 1, *: OR (95% CI) was adjusted for age and sex, [&]Fisher exact test.

Table 4. Genotype distribution of variants between non-surviving and surviving patients at 28-day follow-up.

	Non-surviving group n:9 (%)	Surviving group n:191 (%)	OR Exp (B)	95% CI	p
XRCC4 rs28360071 Genotypes					
D/D	0 (0)	37 (19.4)	1.240[&]	1.157–1.330[&]	0.215[&]
I/D	4 (44.4)	96 (50.3)	1.852*	0.441–7.774*	0.400*
I/I	5 (55.6)	58 (30.3)	0.349[&]	0.090–1.346[&]	0.144[&]
XRCC4 rs6869366 Genotypes					
G/G	9 (100)	146 (76.4)	1.308[&]	1.209–1.415[&]	0.085[&]
G/T	0 (0)	41 (21.5)	1.273[&]	1.182–1.371[&]	0.208[&]
T/T	0 (0)	4 (2.1)	1.021[&]	1.000–1.043[&]	1.000[&]
XRCC1 rs25487 Genotypes					
A/A	4 (44.4)	74 (38.7)	0.791[&]	0.206–3.039[&]	0.738[&]
A/G	5 (55.6)	82 (42.9)	0.602[&]	0.157–2.311[&]	0.506[&]
G/G	0 (0)	35 (18.4)	1.224[&]	1.145–1.309[&]	0.365[&]

XRCC4: X-ray repair cross-complementing protein 4, XRCC1: X-ray repair cross-complementing group 1, *: OR (95% CI) was adjusted for age and sex, [&]Fisher exact test.

3.6. Intensive care and patients in the ward groups

We examined the genotype distribution of the variants by dividing the patients into intensive care and ward patients. There were 16 (8%) people in the intensive care patient group and 184 (92%) in the ward group. There was a significant association in *XRCC4* rs6869366 genotype distribution between the intensive care and inpatient groups. All of the patients hospitalized in the intensive care unit carried the *XRCC4* rs6869366 G/G genotype ($p = 0.025$, OR:1.314, 95%Cl: 1.212-1.425). The genotype distribution of *XRCC4* rs28360071 and *XRCC1* rs25487 did not differ significantly between intensive care patients and inpatients ($p > 0.05$) (Table 5).

Table 5. Genotype distribution of variants between intensive care patients and inpatients.

	Intensive care group n:16 (%)	Inpatient group n:184 (%)	OR Exp (B)	95% CI	p
XRCC4 rs28360071 Genotypes					
D/D	2 (12.5)	35 (19.0)	2.193*	0.412–11.698*	0.358*
I/D	6 (37.5)	94 (51.1)	1.977*	0.613–6.381*	0.254*
I/I	8 (50)	55 (29.9)	0.426[&]	0.152–1.194[&]	0.157[&]
XRCC4 rs6869366 Genotypes					
G/G	16 (100)	139 (75.5)	1.314[&]	1.212–1.425[&]	**0.025**[&]
G/T	0 (0)	41 (22.3)	4.167[&]	0.534–32.507[&]	0.201[&]
TT	0 (0)	4 (2.2)	1.022[&]	1.000–1.044[&]	1.000[&]
XRCC1 rs25487 Genotypes					
A/A	10 (62.5)	68 (36.8)	0.352[&]	0.122–1.011[&]	0.061[&]
A/G	6 (37.5)	81 (44.1)	1.311[&]	0.457–3.757[&]	0.794[&]
G/G	0 (0)	35 (19.1)	1.235[&]	1.151–1.324[&]	0.080[&]

XRCC4: X-ray repair cross-complementing protein 4, XRCC1: X-ray repair cross-complementing group 1, *: OR (95% CI) was adjusted for age and sex, [&]Fisher exact test. The results that are statistically significant are shown in boldface.

4. Discussion

Understanding the mechanism by which SARS-CoV-2 produces systemic and pulmonary damage or the existence of other mechanisms that exacerbate tissue damage in the disease is important. In high-risk patients with pulmonary and systemic inflammation, COVID-19 causes multiple organ dysfunction syndrome.[17] Multiple mechanisms appear to play a role in the complex pathophysiology of COVID-19. A good immune response is required to control this infection, but an inadequate adaptive response as well as a hyperinflammatory response can produce local and systemic tissue damage.[18] In studies, a cytokine storm has been linked to worsening clinical status in patients with COVID-19 infection. It appears to be an important factor in the occurrence of acute respiratory distress syndrome and multiple organ failure.[19]

ROS production in the very early stages of the immune response leads to infected cell death via apoptosis or necrosis.[20] Studies have shown that ROS is a potent ligand and a direct stimulator of the NLR family pyrin domain containing 3 (NLRP3) inflammasome. Furthermore, Toll-like receptors (TLR) and nucleotide binding domain (NBD) and leucine rich repeat related gene family (NLR) ligands increase Nuclear factor kappa B (NF-κB)-mediated transcriptional levels of NLRP3.[21] The infection spreads to the blood as an immune response to OS in the initial phase of virus replication and inflammation. In vitro experiments have shown that SARS-CoV-1 infection increases ROS production in human promonocyte cells and various mammalian cells.[22,23] Violi et al.[24] showed that NADPH oxidase-2 is overexpressed in hospitalized COVID-19 patients, resulting in an increase in OS.[24] Mehri et al.[25] showed that OS markers increased in COVID-19 patients.[25]

DNA, a reactive molecule sensitive to chemical changes, has a repair mechanism to preserve the genomic integrity of cells.[26] Maintaining the integrity of DNA is essential to avoid harmful mutations and maintain the health of the organism.[27] It is well known that OS causes DNA damage. In addition, although RNA viruses complete their life cycle in the host cell cytoplasm, they can induce DNA damage and activate the DNA damage response pathway (DDR). They allow viral replication and modulation in the host cell. Positive-sense RNA viruses from the Coronaviridae family, of which SARS-CoV-2 is a member, and Influenza A viruses from the Orthomyxoviridae family are known to induce the DDR pathway in host cells.[28] Recent studies have shown that SARS-CoV-2 can use the DDR pathway for its spread in host cells.[29] In African green monkey kidney cells, the SARS-CoV-2 infection has been shown to induce DDR.[29]

Damaged DNA is on the path to restoring its integrity with DNA repair enzymes. The integrity, stability, and preservation of the human genome

are crucial to cellular and physiological processes. Its success depends on the DNA repair mechanism. A harmful mutation in DNA repair genes can lead to genomic instability, cancer, and aging. DNA repair gene variants affect the functional properties of DNA repair enzymes, and DNA repair capacity may vary between individuals.[30] Considering the relationship, it is obvious that DNA repair genes have a role in the course of the infection. XRCC4 is involved in non-homologous end-joining (NHEJ) which is an error-prone repair pathway that can occur throughout all cell cycles. It works in conjunction with Ku70/Ku80 and ligase 4 and is important for precision splicing of blunt DNA DSBs. The XRCC4 gene, localized on chromosome 5q14.2 has 23 exons.[31] Variants in the XRCC4 gene have been studied with different cancer types, such as bladder, breast, stomach, oral, and colorectal.[32] *XRCC4* rs6869366 is located in the promoter region of the gene. XRCC4 rs28360071 is a 30 bp deletion/insertion variant in the 3rd intron of the gene. The human *XRCC1* gene is involved in the repair of SBSs breaks from endogenous ROS, alkyl agents, and ionizing radiation. Human *XRCC1* is localized on chromosome 19q13.2 and is composed of 17 exons.[33] Lunn et al.[34] reported that the interaction of rs25487 in the BRCA1 C-terminal (BRCT) domain with Poly (ADP-ribose) polymerase (PARP) may lead to defects in DNA repair.[34] It has been reported that the *XRCC1* rs25487 G allele has a high probability of having detectable polyphenol DNA adducts and is associated with smoking addiction. Sister chromatid exchange was common in subjects carrying the *XRCC1* rs25487 G allele.[33]

In this study, we examined the susceptibility of three variants involved in the DNA repair mechanism to COVID-19. We compared the distribution of these three variant genotypes and alleles between patients and controls. We found that the *XRCC4* rs6869366 variant G/G genotype and G allele were associated with disease occurrence. *XRCC1* rs25487 A/A and A/G genotypes and the A allele frequency were found to be higher in patients than controls. Genotype and allele distribution of the *XRCC4* rs28360071 variant did not differ between patients and controls (Table 2). We then evaluated whether these variants have an impact on the clinical course of the disease. We divided the patient group into three groups based on clinical characteristics. When we divided the cases into severe and mild infections, no difference was found in the genotype distribution of the three variants (Table 3). Similarly, the genotype distribution did not differ between the groups that did not survive and those that survived the 28 follow-up periods (Table 4). Comparing the patients in need of intensive care with those hospitalized in the ward, all of the patients hospitalized in the intensive care unit had the *XRCC4* rs6869366 G/G genotype.

This study had some limitations. The relatively small patient group, when divided into clinical subgroups, may have affected the statistical significance; therefore, it may be necessary to create larger patient groups. We focused on only three variants in the study. There are also different gene variants involved in the DNA repair mechanism.

5. Conclusion

To our knowledge, this is the first study to report DNA repair gene variants in COVID-19 patients. Our results suggested that *XRCC4* rs6869366 and *XRCC1* rs25487 were associated with COVID-19 susceptibility and clinical course. The short- and long-term effects of the global SARS-CoV-2 pandemic on human health still remain unclear. It is not clear exactly what diseases they will encounter in the future in recovered COVID-19 patients. Coronaviruses can cause DNA damage and destabilize the genome by disrupting DNA repair mechanisms. Understanding the etiopathogenesis of the disease will guide the measures to be taken.

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Declarations

Availability of data and materials

Data are available upon request to the corresponding author. The authors declare that data supporting the findings of this study are available within the referenced articles.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CRediT authorship contribution statement

SP conceived the study; SP, GYS, MK, AM, AA, NS, HK, UIA, TC, HK, TT and IS acquired data; SP analyzed data; NS and IS wrote the original draft; all authors revised and approved the final manuscript.

Ethics approval and consent to participate

Ethical committee approval was received (Istanbul University, Faculty of Medicine, approval date and number: 21/05/2020-84539) and the patients and control subjects gave written informed consent before the beginning of the study. The experimental procedures were based on the Declaration of Helsinki and relevant institutional regulations.

Patient consent for publication

Informed consent was obtained in written form from all of the patients to publish this work.

Informed consent

Informed consent was obtained from all individual participants included in this study.

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