Do Repeated Episodes in Unipolar Depression Affect the DNA Damage, Repair Mechanisms and Cognitive Functions?

Mustafa Metehan Yıldırım, MD,[1] Osman Zulkif Topak, MD,[1] Osman Ozdel, MD,[1] Ibrahim Acikbas, MD,[2] and Mucahit Secme, PhD[2]

[1]*Pamukkale University, Department of Psychiatry, Denizli, Turkey*
[2]*Pamukkale University, Department of Medical Biology, Denizli, Turkey*

ABSTRACT

Background: Memory impairments seen in depression have a significant role in daily functionality and work performance. In the pathogenesis of commonly seen and incapacitating diseases such as depression there is a need to clarify points that remain unknown.

Aim: The aim of this study was to investigate the relationship between DNA damage, repair efficiency and cognitive functions in single episode and repeated episodes of depressive disorder.

Methods: The study included 40 patients with a single episode of depression, 38 with repeated episodes and 40 healthy control subjects. DNA damage was examined using the comet assay method, and levels of OGG1, NEIL1, XRCC1 and APEX1 gene expression were measured using the real-time PCR method. The Verbal Memory Process Test was applied to all participants. The Hamilton Depression Rating Scale and Clinical Global Impression were also applied to the patient groups.

Results: In the group with recurrent depression, it was observed that as the number of episodes increased and the duration of the disease lengthened, so the DNA damage increased. As DNA damage increased, so memory functions were observed to be impaired. DNA damage was associated with the decrease in the levels of DNA repair genes APEX1 and OGG1. The APEX 1 gene expression levels were determined to be reduced in the repeated depression group.

Conclusions: The study results showed that as the number of depressive episodes increased, the effectiveness of DNA repair decreased and DNA damage increased, and the memory impairments seen in recurrent depression could be associated with DNA damage.

INTRODUCTION

Major depressive disorder is a mood disorder that progresses with impairments in psychophysiological functions and clinically causes significant distress, impaired functionality and disability (1). As it has a heterogenous course, some patients only ever experience a single depressive period during their lifetime, and others may experience repeated episodes (2). Following each episode, the prognosis is worse and there is usually less response to pharmacological treatment (3).

In the pathogenesis of commonly seen and incapacitating diseases such as depression there is a need to clarify

Declaration of Competing Interest and Source of Funding

The authors declared have no conflicts of interest with respect to their authorship or the publication of this article.
This study was supported by the Pamukkale University Scientific Research Projects Coordination Unit (Project number: 2019TIPF009).

Approval

All patients provided written informed consent for participation in the study. Approval for the study was granted by the Pamukkale University's Non-Interventional Clinical Investigations Ethics Committee dated 14.11.2018 and numbered 60.116.787-020 / 77477.

Address for Correspondence: ✉ Osman Zulkif Topak, MD, Pamukkale University, Department of Psychiatry, Kınıklı Campus, Pamukkale, Denizli, 20160 Turkey drosmantpk@hotmail.com

points that remain unknown. Although there have been many studies in this field, the pathogenesis and etiology of depression remain elusive. Therefore, the molecular aspects of the disease should be extensively studied. Inflammation and increased oxidative stress are thought to play a significant role in the pathogenesis of major depressive disorder (4). Increased oxidative stress is known to cause damage in biomolecules, including DNA (5), and DNA damage has emerged as one of the leading components in the etiology of several neuropsychiatric diseases such as depression, bipolar disorder, and schizophrenia (6, 7). This knowledge has revealed the need to study DNA damage processes. In a meta-analysis that examined DNA damage in depression, 8 OHdG, a marker of DNA damage, was shown to be increased in depressed patients (8). However, the majority of studies which have focussed on depressive symptoms and oxidative DNA damage have shown differing results of the evaluation of DNA damage by measurement of 8-OHdG nucleoside due to the sample types and measurement methods used (8). The single cell gel electrophoresis Comet Assay test is a rapid, sensitive, and quantitative technique to determine DNA damage in cells (9). In the comet assay method, measurements are made on the image of the comet-shape formed by DNA fractures migrating from the cell nucleus (10).

However, cells have various DNA repair mechanisms to protect genomic integrity. Over the last two decades, there has been increasing evidence that the DNA damage occurring in the course of depression is not only the result of oxidative stress but that impairments in DNA repair also contribute to the increased DNA damage (7). In a previous study by the current authors, a high rate of DNA damage was determined in schizophrenia patients, and it was concluded that in addition to high rates of oxidative stress, this could be due to insufficient DNA repair capacity (11). Polymorphisms in genes involved in DNA repair, such as OGG1 (8-oxoguanine DNA glycosylase), NEIL1 (Nei-like DNA glycosylase), X-ray repair complementing group 1 (XRCC1) and APEX 1 (apurinic/apyrimidinic endonuclease) can change the activity of proteins and the repair capacity. An inadequate repair capacity is a substantial contributing factor to genetic instability (12). OGG1 repairs 8-OHG (8-hydroxyguanosine), which is one of the most important biomarkers for oxidative DNA damage (13). Studies have indicated that OGG1 expression is highest in the brain (14). NEIL1, which is found in almost every tissue, is mostly found in human tissues such as the thymus, pancreas, liver, kidney, muscle, intestine and brain (15). XRCC1 is responsible for the repair of BER (Base Excision Repair), single strand breaks and DNA proteins such as DNA polymerase and DNA ligase III. Damage for which the BER DNA repair mechanism is responsible include ionizing radiation, alkylating agents and oxidation (16). APEX 1 is an important enzyme in the DNA repair mechanism. A deoxyribose sugar that has lost its purine and/or pyrimidine base is recognized by the enzyme APEX1, the enzyme cuts the phosphodiester bone and removes the damaged area. Then the damage begins to be repaired with the help of a phosphodiesterase (17).

Another noteworthy point in major depressive disorder is that there are cognitive defects in addition to mood and behavioral symptoms in the disease (18). These cognitive defects have a significant role in daily functionality and work performance (19). Previous studies have shown that repeated episodes of major depressive disorder create loss in the neurocognitive area (20). Oxidative stress may also play a role in cognitive decline and can cause memory failure in particular (21).

The aim of this study was to determine DNA damage and repair mechanisms in patients with a single episode and repeated episodes of major depressive disorder, and to assess the effect of DNA damage and the repair capacity on memory functions. Cognitive deficits in depression constitute a notable component of the burden of the disease and there is need to determine the etiology. The study hypothesis was that DNA damage may play an important role in cognitive deficits in depression.

SUBJECTS AND METHOD

The study included 40 patients with single episode depression and 37 with recurrent depression, diagnosed according to DSM-5 at the Pamukkale University Medical School Department of Psychiatry. Each patient was literate, with normal mental capacity, was aged between 18 and 60 years, with no physical or neurological disease. A control group was formed of 40 healthy volunteers, matched in age and smoking habits, literate, with normal mental capacity, with no physical/ neurological or psychiatric disease and taking no medication.

Each participant was informed about the study, and written informed consent was obtained in accordance with the Helsinki Declaration. Prior to the research, approval was received from University Non-Interventional Clinical Investigations Ethics Committee.

A socio-demographic data form was completed for each participant. The structured clinical interview form

(SCID-5) for DSM (22) and the Verbal Memory Process Test (VMPT) were also applied to all participants (23). The Hamilton Depression Rating Scale (HAM-D) (24) and Clinical Global Impression (CGI)(25) were also applied to the patient groups. To evaluate the relationship between severity of the disease and DNA damage, the patients were selected from referrals, the walk-in clinic, and inpatients.

The amount of 6ml venous blood was obtained from each participant for analysis. DNA damage was measured in lymphocytes using the comet assay method; OGG1 (8-oxoguanine DNA glycosylase), NEIL1 (Nei-like DNA glycosylase), APEX1 (Apurinic/Apyrimidinic Endodeoxyribonuclease 1) and XRCC1 (X-Ray Repair Cross Complementing 1) gene expressions were obtained with real-time PCR. The blood samples were analyzed immediately after being received and were studied in the dark so that they were minimally affected by environmental factors. To minimize the effects of diet, fasting blood samples were obtained.

COMET ASSAY

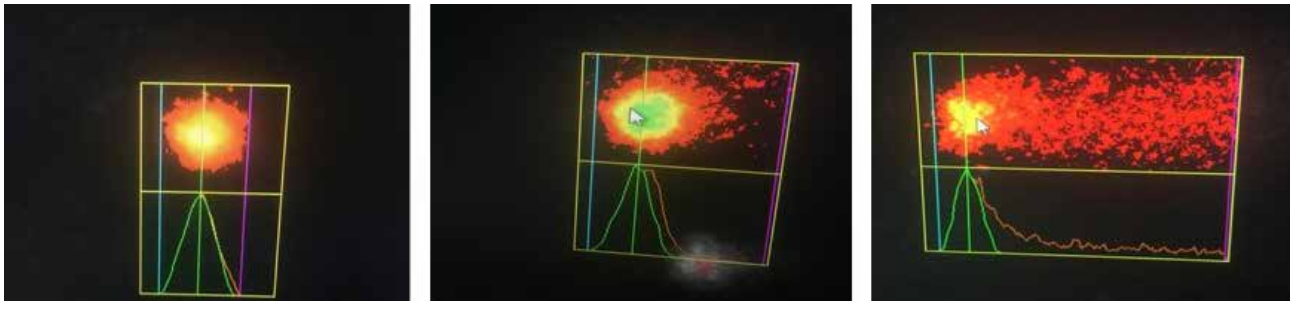
The comet assay is a quantitative method for determining the DNA damage in lymphocyte cells. The working procedure for this assay in this study was as follows. Lymphocytes were isolated using Histopaque (Sigma) with Leucosep™ Centrifuge Tubes. The cells were suspended in 0.1M PBS (20 000 cells in 25µl PBS). Three frosted slides per sample were prepared by adding three layers of low melting point agarose gel (LMPA, 37°C). Once solidified, all slides were immersed in freshly prepared cold lysing solution [2.5M NaCl, 100mM EDTA, 1% Triton X-100, 10mM Tris (pH 10) and 10% DMSO] and incubated (1 hr., 4°C). Following incubation, the slides were placed in an electrophoresis buffer [0.3 M NaOH, 1mM Na2EDTA; pH 13] for 20 min at 4^0C. The slides were then electrophoresed (25V (300mA, approx. 0.74V/cm) for 35 min at 4^0C. Then, the slides were washed for 5 mins three times with a neutralization buffer [0.4M Tris; pH 7.5]. After this step, the slides were plunged into methanol for five minutes at -20 ^{0}C, then placed on a smooth surface and dried. Before the examination, the slides were stained with ethidium bromide (40 µL), and were then viewed using a Nikon fluorescent microscope with 510-560nm excitation and 590nm emission filters. Images of at least 50 randomly selected comets on each triplicate slide were captured per sample at x20 magnification, and image analyses were performed using the Comet Assay IV Version 4.3.2 for Basler FireWire and reported as µm.

Broken DNA molecules move at different rates in electrical fields as they have different molecular weights and electric charges. Damaged fragments of DNA moving towards the anode present a comet-like image, but intactus DNA cannot come out of the helical structure. The parameters used to measure the damage were HL (Head Length, µm), TL (Tail Length, µm) HD (Head Density: percentage of DNA in head), TD (Tail Density: percentage of DNA in tail), and TMo (Tail Moment, expressed in µm, is the value obtained by multiplying TL and TD then dividing by 100). As the DNA damage increases, head length increases, head density decreases, and the tail length, tail density, and tail moment increase (Figure 1).

RNA ISOLATION AND REAL TIME PCR

RNA isolation was performed from nucleated blood cells, cDNA synthesis was performed and the gene expression changes of OGG1, NEIL1, XRCC1, APEX1 genes were compared between the control group and patient groups with Real-Time PCR. Total RNA was isolated from the lymphocyte cells using Trizol Reagent (Ambion) according to the manufacturer's instructions. cDNA synthesis from the RNA template was performed via reverse transcription using the Transcriptor High Fidelity

Figure 1. *Comet Images: Images of increasing DNA damage*



cDNA Synthesis Kit (CatNo: 05 081 955 001) according to the manufacturer's protocols. Gene expression analysis was performed using Step One Plus Real Time RT-PCR (Applied Biosystems, U.S.A.) according to the SYBR Green qPCR Master Mix (Thermo Scientific, U.S.A.) protocol. The RT-PCR assay was performed using gene-specific primers. The expression results were regulated to the *beta-actin* gene (housekeeping gene) expressions to calculate relative expression ratios. The forward and reverse sequences of these genes were designed using OriGene (https://www.origene.com/) online web page and BLAST (Basic Local Alignment Search Tool) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) software.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS 25.0 software (IBM SPSS Statistics 25 software Armonk, NY, U.S.A.). Continuous variables were expressed as mean ± standard deviation (SD), median (minimum-maximum) values, and categorical variables were expressed as number (n) and percentage (%). The normality of data was examined with the Kolmogorov Smirnov and Shapiro Wilk tests. Comparisons of independent groups were made. The Student's t-test and One-Way ANOVA were used when the parametric test assumptions were satisfied (Post hoc: "Tukey Test"), and when parametric test assumptions were not satisfied, the Mann-Whitney U test and the Kruskal-Wallis Analysis of Variance were used (Post hoc: "Bonferroni Correction"). Bonferroni correction was performed to avoid type 1 error and the level of significance was determined by dividing the p-value (0.05) into the number of pairwise comparisons. Chi-square analysis was used to compare categorical variables and Spearman correlation analysis to evaluate the relationships between continuous variables. The level of (p) 0.05 was used to determine statistical significance.

RESULTS

CLINICAL AND SOCIODEMOGRAPHIC DATA

The sociodemographc data of all the study groups are shown in Table 1. No significant difference was determined between the groups in respect of age, gender, marital status, place of residence, occupational status, body mass index, smoking status, or alcohol consumption. In comparison with the control group, a history of migration was found to be at a higher rate and the duration of education was found to be shorter in the patient group.

Table 1. *Sociodemographic Characteristics of Groups*

	Single Episode Depression n (%) Mean (±sd)	Recurrent Depression n (%) Mean (±sd)	Control n (%) Mean(±sd)	p
Age	31.63 ± 11.4	35.73 ± 12.65	34.68 ± 11.03	0.318
Gender				
Female	28 (70)	29 (78,4)	27 (67,5)	0.543
Male	12 (30)	8 (21,6)	13 (32,5)	
Marital status				
Maried	22 (55)	20 (54,1)	24 (60)	0.475
Single	17 (42.5)	13 (35.1)	15 (37.5)	
Divorced	1 (2.5)	4 (10.8)	1 (2.5)	
Living				
Urban	33 (82.5)	27 (73)	37 (92.5)	0.075
Rural	7 (17.5)	10 (27)	3 (7.5)	
Migration Status				
İmmigrant	5(12.5)	6 (16.2)	0 (0)	0.007*
Not İmmigrant	35 (87.5)	31 (83.8)	40 (100)	
Education Time	10.15 ± 4.33	10.41 ± 4.04	12.55 ± 3.54	0.013*
Working Status				
Employed	18 (45)	16 (43.2)	27 (67.5)	0.056
Unemployed	22 (55)	21 (56.8)	13 (32.5)	
BMI Mean(ss)	23.81 ± 4.15	25.9 ± 6.44	24.97 ± 5.3	0.394
Cigarette				
Yes	22 (55)	22(59.4)	23 (57.5)	0.924
No	17 (42.5)	13 (35.1)	13 (32.5)	
Quit	1 (2.5)	2 (5.5)	4 (10)	
Cigarette (Packs/ Year)	5.49 ± 9.75	5.18 ± 10.38	4.63 ± 8.23	0.856
Alcohol				
1-2 per week	2 (5)	2 (5.4)	4 (10)	0.825
Rarely	6 (15)	6 (16.2)	4 (10)	
No	32 (80)	29 (78.4)	32 (80)	

*p<0.05 BMI : Body Mass Index

In the group with repeated episodes of depression, the mean number of episodes was 3.03±1.26. When the patient groups were evaluated according to the clinical characteristics, a familial history of depression, hospitalization history and history of suicide attempts were determined at significantly higher rates in the patients with repeated episodes of depression. The HAM-D points showed no difference between the groups, and the CGI points were determined to be significantly higher in the patients with repeated episodes of depression. The disease was determined to have started at an earlier age in the patients with repeated epidsodes. No difference was determined between the groups in terms of the medical treatments (Table 2).

NEUROCOGNITIVE EVALUATION

The neurocognitive evaluations of the study participants are summarized in Table 3. The short-term memory recall

Table 2. *Clinical Characteristics of Patients*

		Single Episode Depression n (%)	Recurrent Depression n (%)	p
Depression History of Family	No Yes	32 (80) 8 (20)	15 (40.5) 22 (59.5)	0.000*
Hospitalization History	No Yes	36 (90) 4 (10)	20 (54.1) 17 (45.9)	0.000*
Suicide History	No Yes	36 (90) 4 (10)	24 (64.9) 13 (35.1)	0.008*
EKT History	No Yes	40 (100) 0 (0)	34 (91.9) 3 (8.1)	0.106
Age of onset of the disease		31.18 ± 11.34	25.19 ± 9.16	0.011*
HAM-D		18.98 ± 5.58	20.32 ± 3.79	0.119
CGI		4.25 ± 0.71	4.62 ± 0.64	0.017*
AD		11 (27.5)	13 (35.1)	0.470
AD + AP		6 (15)	8 (21.6)	0.452
AD+MS		0 (0)	3 (8.2)	0.106
No Medication		23(57.5)	13(35.1)	

* p<0.05 AD:Antidepressant AP:Antipsychotics MS: MoodStabilizer

Table 3. *Comet Values, DNA Repair Genes and Cognitive Functions of the Groups*

	Single Episode Depression	Recurrent Depression	Control	p
Head Length	71.82±16.16	72.14±13.99	73.54±15.08	0.732
Tail Length	74.92±15.62	79.93±13.98	74.75±14.86	0.179
Head Density	77.55±12.86	78.98±12.72	70.17±12.25	0.004*
Tail Density	22.27±12.78	21.22±13.00	29.82±12.25	0.004*
Tail Moment	12.09±14.64	10.51±9.96	15.25±10.57	0.027*
OGG1	27.14 ± 4.3	26.34 ± 3.9	28.06 ± 4.36	0.225
NEİL1	31.8 ± 2.64	31.23 ± 2.33	31.65 ± 3.71	0.288
XRCC1	28.66 ± 3.92	27.51 ± 3.87	29.62 ± 5.36	0.118
APEX1	27.71 ± 4.24	26.7 ± 4.25	29.12 ± 4.03	0.042*
IM	5.33 ± 1.73	4.95 ± 1.61	5.85 ± 1.39	0.062
STM-SR	13.38 ± 2.37	12.65 ± 2.78	14.55 ± 1.48	0.000*
LTM-SR	11.23 ± 2.41	10.32 ± 2.54	11,91 ± 1.86	0.017*
LS	106.08 ± 20.15	101.68 ± 21.59	120.48 ± 13.19	0.000*

* p<0.05 IM: Immediate Memory, STM-SR: Short-Term memory spontaneously-recall score, LTM-SR: Long-term memory spontaneously-recall score, LS: Learning Score. (Table showing more damage, inadequate DNA repair and poor neurocognitive functions in the recurrent depression)

points and the learning points of the control group were significantly higher than those of both the single episode and recurrent depression groups. The long-term memory recall points were determined to be significantly higher in the control group than in the recurrent depression group. Neurocognitive functions were observed to be poor in the recurrent depression group.

DATA RELATED TO DNA DAMAGE AND REPAIR GENES

The groups were evaluated using comet analysis. The findings are summarized in Table 3. No statistically significant difference was determined between the single episode depression group and the recurrent depression group in respect of the level of DNA damage. The level of APEX1 gene expression was determined to be significantly higher in the control group than in the recurrent depression group.

In the single episode depression group and control group, there was no difference between the genders in respect of DNA damage (single episode; HL: p=0.131; TL: p=0.799; HD: p=0.393; TD p=0.389; TM p= 0.443. control group; HL p=0.778; TL p=0.178; HD p=0.928; TD p=0.928; TM p= 0.761). In the recurrent depression group the DNA damage was determined to be greater in males (HL p=0.508; TL p=0.262; HD p=0.067; TD p=0.094; TM p= 0.047).

In the single episode depression group, DNA damage was seen to increase with cigarette smoking (non-smokers HL: median 66.05, interquartile difference 11.37; smokers HL:median 79.35, interquartile difference 18.74, p= 0.010). No relationship was determined between DNA damage and alcohol consumption. In the recurrent depression group and the control group, no relationship was determined between DNA damage and cigarette smoking or alcohol consumption (p>0.05)

When the relationship was examined between DNA damage and the medications used by the patients, no difference was determined according to the groups of antidepressants (AD), AD+ antipsychotics (AP), and AD+ mood stabilizers (MS) (p>0.05). The relationship with DNA damage and the most used medications was also examined. No relationship was determined between DNA damage and the use of sertraline, fluoxetine and quetiapine (p>0.05). DNA damage was seen to be increased with the use of venlafaxine (HL: group using venlafaxine: median 81.28, interquartile difference 18.98; group not using venlafaxine: median 70.23, interquartile difference 13.67, p=0.048) (TM: group using venlafaxine: median 7.30, interquartile difference 4.1; group not using venlafaxine: median 12.07, interquartile difference 12.86, p=0.048).

Spearman correlation analysis was applied to examine the relationships between DNA damage and the sociodemographic data and clinical characteristics of the groups. No significant correlation was determined between DNA damage and age, body mass index, smoking status, age at onset of disease, duration of or severity of disease

(HAM-D, CGI) (p>0.05). A negative correlation was determined between HD and the number of episodes in the recurrent depression group (p=0.045, r=-0.331), and a positive correlation was determined between TM and the number of episodes (p=0.019, r=0.383) and between TL and TM and the duration of the disease (TL: p=0.017; r= 0.391; TM: p=0.016; r= 0.393). These findings showed that as the duration of the disease lengthened and the number of episodes increased, so DNA damage increased.

The correlations between the cognitive functions of the patients and DNA damage and repair mechanisms are shown in Table 4. In the recurrent depression group, a negative correlation was determined between long-term memory spontaneous recall points and TL. This showed that when DNA damage increased, the long-term memory points decreased. A positive correlation was determined between OGG1 gene expression levels and HD and a negative correlation was determined with TD. APEX1 gene expression levels were also determined to be positively correlated with HD, and negatively correlated with TD. These results showed that as the OGG1 and APEX1 gene expression levels increased, DNA damage decreased.

DISCUSSION

This study was conducted to investigate DNA damage in the pathophysiology of single episode and recurrent depression, DNA repair mechanisms and the effects of these on memory functions. The results of the study showed no difference between patients with a single episode or repeated episodes of depression in respect of DNA damage. However, the most important finding of the study was that as the number of depressive episodes increases, the efficacy of DNA repair diminishes, so DNA damage is increased and this increase in DNA damage was seen to be associated with impaired memory functions. There are studies in literature that have investigated DNA damage and repair mechanisms in depressive disorders, but to the best of our knowledge there is no previous study which has evaluated the effect on cognitive functions of the difference in DNA damage and repair mechanisms between single episode and recurrent depression groups of patients. Thus, this is the first study to have shown that the memory impairments seen in recurrent depression could be related to the increase in DNA damage.

In research made with the comet technique, it has been reported that chronic diseases and advanced age affect DNA damage (26). Therefore, patients aged over 60 years and those with additional physical or neurological diseases were not included in the current study to provide reliability of the results, and ensure similar mean ages of the groups. It has been reported that smoking is one of the exogenous sources that cause free radicals and conditions such as smoking and alcohol consumption

Table 4. *Correlation of DNA Damage with Repair Gene Expressions and Cognitive Functions*

		Single Episode Depression					Recurrent Depression				
		HL	TL	HD	TD	TM	HL	TL	HD	TD	TM
IM	p	0.138	0.605	0.220	0.226	0.192	0.364	0.261	0.138	0.124	0.372
	r	0.242	-0.085	0.201	-0.198	-0.213	0.154	-0.190	0.248	-0.257	-0.151
STM-SR	p	0.835	0.663	0.995	0.909	0.879	0.533	0.204	0.673	0.707	0.644
	r	-0.034	0.072	-0.001	0.019	0.025	-0.106	-0.214	0.072	-0.064	-0.079
LTM-SR	p	0.669	0.686	0.982	0.928	0.784	0.864	**0.026***	0.982	0.928	0.784
	r	-0.071	-0.067	-0.004	0.015	-0.045	-0.071	**-0.366***	0.206	-0.200	-0.246
LS	p	0.824	0.750	0.662	0.607	0.802	0.993	0.063	0.266	0.280	0.349
	r	-0.037	0.053	-0.072	0.085	0.041	-0.001	-0.309	0.188	-0.182	-0.159
Ogg-1	p	0.381	0.610	0.639	0.638	0.988	0.064	0.094	**0.011***	**0.012***	0.077
	r	-0.144	-0.084	-0.078	0.078	-0.002	0.307	-0.280	**0.413***	**-0.408**	-0.294
Neil-1	p	0.97	0.783	0.436	0.439	0.802	0.751	0.537	0.279	0.266	0.823
	r	-0.001	0.046	-0.128	0.128	0.041	0.054	0.105	-0.183	0.188	0.038
Xrcc-1	p	0.797	0.904	0.568	0.563	0.849	0.193	0.472	0.134	0.151	0.375
	r	-0.043	0.020	-0.094	0.096	0.032	0.219	-0.122	0.251	-0.241	-0.150
Apex-1	p	0.714	0.735	0.533	0.527	0.873	0.125	0.164	**0.012***	**0.016***	**0.031***
	r	-0.061	0.056	-0.103	0.104	0.027	0.257	-0.234	**0.407***	**-0.394***	**-0.356***

* p<0.05 IM: Immediate Memory, STM-SR: Short-Term memory spontaneously-recall score, LTM-SR: Long-term memory spontaneously-recall score, LS: Learning Score. (Table showing that as DNA damage increased, the long-term memory points decreased; and as the OGG1 and APEX1 gene expression levels increased, DNA damage decreased.)

can cause DNA damage (27, 28). In the current study, as there was no statistically significant difference between the groups in respect of age, gender, body mass index, smoking and/or alcohol consumption, marital status, place of residence, and occupational status, it can be said that the reliability of the results was increased by limiting the environmental and psychosocial factors that could affect DNA damage. Another parameter affecting DNA damage is the drugs used by patients. In literature there are studies with conflicting findings as to whether antidepressants increase or decrease DNA damage (29). Similarly, several studies have shown that antipsychotics and mood stabilizers used in the treatment of patients with depression have different effects on DNA damage and repair (30-32). In the current study, the group distribution was similar when the single episode and repeated episodes depression groups were categorized according to the type of medications used, which also contributed to minimizing the factors affecting DNA damage.

Several studies have reported that DNA damage is increased in patients with recurrent depressive disorder (5, 33). It has been shown that serum levels of 8-OHdG, which is a marker of DNA damage, are higher in patients with repeated episodes of depression and as the number of episodes increases so the DNA damage increases (34). However, there are also different results in literature. In a previous study, the oxidant/antioxidant balance that reacts with DNA and causes damage was measured in single and repeated episodes depression groups, and the antioxidant enzyme levels were examined, such as manganese superoxide dismutase, myeloperoxidase, nitric oxide synthase and cyclo-oxygenase. No significant difference was determined between the groups in respect of the parameters causing DNA damage similar to the current study results (35).

In the current study, although no difference was determined in respect of DNA damage between the patients with a single episode of depression and those with recurrent depression, it was seen from the correlation analyses that as the number of episodes increased, so the DNA damage increased. Considering that the patient is exposed to the effects of the disease for longer with an increased number of episodes and a longer duration of follow-up, DNA damage may be a result of the destructive effects of the disease, but it may also be due to the inadequate repair mechanisms. The level of APEX1 expression from the DNA repair genes was found to be low in the repeated episodes depression group.

When the literature related to DNA repair is examined, there can be seen to be conflicting results. In a previous study, it was reported that NLRP3 concentration, which is an inflammatory marker, was increased in depression and this caused the triggering of apoptosis via the p53 pathway by suppressing the DNA repair mechanisms (36). Czarny et al. (33) determined that DNA repair functioned less in a recurrent depression group than in a control group. However, there are also studies indicating that DNA repair is increased in depression. This may also be a balance mechanism which develops as a response to increased DNA damage. In a study of acute leukaemia patients (37), an increase was determined in OGG1 expressions in patients with depressive symptoms compared to those without depressive symptoms and the control group. In another study (38), an increase was determined in both DNA damage and OGG1 expression levels in gastric adenocarcinoma patients with depressive symptoms compared to those without. In a postmortem study of depression patients (39), it was observed that PARP1 and OGG1 levels were elevated in the brain white matter oligodendrocytes, and this was thought to be a compensatory mechanism developing secondary to increased DNA damage. In the current study, the level of APEX1 was determined to be significantly low in the recurrent depression group compared to the control group. The OGG1, NEIL1, and XRCC1 levels were determined to be lower in the recurrent depression group than in the control group, but not to a statistically significant level probably because of the small sample size. This could also have been because the patients with recurrent depression were not all selected from hospitalized patients and the disease severity was low. In other studies where DNA repair has been seen to be high in depression there have been observed to be severe comorbid diseases such as cancer included in the sample, whereas in the current study, patients with a chronic disease were excluded. In this respect, the results of the current study can be said to be more reliable.

There have been shown to be deteriorations in cognitive areas in depression, and repeated episodes have been shown to create loss in the neurocognitive area (18, 20). Consistent with findings in literature, the cognitive functions of the patient groups were observed to be statistically significantly low in the current study. Cognitive defects in depressive disorder are known to have an inhibitory effect on daily functionality and work performance, so when the duration and frequency of depressive episodes increase, cognitive impairments may become permanent and even if depressive symptoms recover, the cognitive impairments may persist (40).

It has been reported that oxidative stress could play a role in the etiology of cognitive defects in depressive disorder (21, 41, 42) or that they could be related to hippocampal metabolism changes or decreased hippocampal volume (40, 43). In the current study, the determination of decreased long-term memory recall points as DNA damage increased and that DNA damage increased as the number of episodes increased suggested that DNA damage could have a role in memory impairment. There are studies in literature related to oxidant-antioxidant systems and memory (35, 44). However, to the best of our knowledge, this is the first study to have shown the relationship between DNA damage and memory functions. The specificity of these findings is that there was seen to be a relationship between DNA damage and cognitive deficits. This raises the question of whether some protective measures against DNA damage could delay the onset of disabilities in severe psychiatric disorders in the future.

There were some limitations to this study. Less frequently used classes of drugs were not evaluated in this study. Furthermore, limiting the drug selection to the last four weeks of treatment and the lack of scale to evaluate drug compliance were also limitations. The influence of nutritional habits, lifestyle, exercise, and other nonspecific factors on DNA damage cannot be disregarded. Fasting blood samples were taken to minimize the effect of diet, but it was not possible to completely homogenize the groups in terms of nutritional habits. The lack of a mechanism for measuring such genotoxic variables, and the small sample group in terms of influencing the strength of statistical dependence can also be said to be limitations of this study. Further studies with larger sample size will help to clarify this issue.

In conclusion, the results of this study showed that as the number of depressive episodes increases, DNA damage increases and the effectiveness of DNA repair decreases, and the memory impairments seen in recurrent depression may be associated with DNA damage.

References

1. Kaplan BJ, Sadock VA, Ruiz P. Kaplan & Sadock's synopsis of psychiatry: Behavioral sciences/Clinical psychiatry, 11th ed. Lippincott, New York: Wolters Kluwer Health, 2015.
2. Patten S. Recurrence risk in major depression. Depress Anxiety 2013;30:1-4.
3. Richardson R, Richards DA, Barkham M. Self-help books for people with depression: A scoping review. J Mental Health 2008;17: 543-552.
4. Pasco JA, Nicholson GC, Williams LJ, et al. Association of high-sensitivity C-reactive protein with de novo major depression. Br J Psychiatry 2010;197:372-377.
5. Czarny P, Kwiatkowski D, Kacperska D, et al. Elevated level of DNA damage and impaired repair of oxidative DNA damage in patients with recurrent depressive disorder. Med Sci Monit 2015; 21:412-418.
6. Suberbielle E, Sanchez PE, Kravitz AV, et al. Physiologic brain activity causes DNA double-strand breaks in neurons, with exacerbation by amyloid-β. Nat Neurosci 2013;16: 613-621.
7. Czarny P, Bialek K, Ziolkowska S, et al. DNA damage and repair in neuropsychiatric disorders. What do we know and what are the future perspectives? Mutagenesis 2020;1:79-106.
8. Black CN, Bot M, Scheffer PG, et al. Is depression associated with increased oxidative stress? A systematic review and meta-analysis. Psychoneuroendocrinology 2015;51:164-175.
9. Ostling O, Johanson KJ. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem Bio Res Commun 1984;123: 291-298.
10. Nickson CM, Parsons JL. Monitoring regulation of DNA repair activities of cultured cells in-gel using the comet assay. Front Genet 2014; 5: 232.
11. Topak OZ, Ozdel O, Dodurga Y, Secme M. An evaluation of the differences in DNA damage in lymphocytes and repair efficiencies in patients with schizophrenia and schizoaffective disorder. Schizophr Res 2018;202: 99-105.
12. Ekmekci A, Konac E, Önen Hİ. Gene polymorphism and genetic susceptibility to cancer. Marmara Medical J 2008;21: 282-295.
13. Boiteux S, Radicella JP. Excision repair of 8-oxoguanine in eukaryotes. In: M Dizdaroglu, AE Karakaya, editors. Advances in DNA damage and repair: Oxygen radical effects, cellular protection, and biological consequences, first ed. NewYork: Kluwer Academic/Plenum, 1999: pp. 35-45.
14. Milton NG. Role of hydrogen peroxide in the aetiology of Alzheimer's disease. Drugs & Aging 2004;21: 81-100.
15. Stadtman E, Levine R. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 2003;25:207-218.
16. Chacko P, Rajan B, Joseph T, et al. Polymorphisms in DNA repair gene XRCC1 and increased genetic susceptibility to breast cancer. Breast Cancer Res Treat 2005;89:15-21.
17. Chou K-M, Cheng Y-C. An exonucleolytic activity of human apurinic/apyrimidinic endonuclease on 3′ mispaired DNA. Nature 2002;415:655.
18. Kessler RC, Berglund P, Demler O, et al. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey replication. Arch Gen Psychiatry 2005;62: 593-602.
19. Kessler RC, Akiskal HS, Ames M, et al. Prevalence and effects of mood disorders on work performance in a nationally representative sample of US workers. Am J Psychiatry 2006;163:1561-15618.
20. Slyepchenko A, Maes M, Köhler CA, et al. T helper 17 cells may drive neuroprogression in major depressive disorder: Proposal of an integrative model. Neurosci Biobehav Rev 2016;64:83-100.
21. Evola M, Hall A, Wall T, et al. Oxidative stress impairs learning and memory in apoE knockout mice. Pharmacol Biochem Behav 2010;96:181-186.
22. Elbir M, Alp Topbaş Ö, Bayad S, et al. Adaptation and reliability of the structured clinical interview for DSM-5 disorders - Clinician version (SCID-5/CV) to the Turkish language. Turk Psikiyatri Derg 2019;30: 51-56.
23. Öktem Tanör Ö. Öktem Verbal Memory Process Test (ÖKTEM SBST) Hand book, 1st ed. Ankara: Türk Psikologlar Derneği Publications, 2011.
24. Akdemir A, Turkcapar MH, Orsed SD, et al. Reliability and validity of the Turkish version of the Hamilton Depression Rating Scale. Compr Psychiatry 2001;42:161-165.
25. Guy W. Clinical Global Impression (CGI). In AJ Rush, editor. Handbook of Psychiatric Measures. Washington DC: American Psychiatric Association, 2000: pp. 100-102.
26. Møller P, Knudsen LE, Loft S, Wallin H. The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents

and effect of confounding factors. Cancer Epidemiol Biomarkers Prev 2000;9:1005-1015.

27. Thomas MJ. The role of free radicals and antioxidants. Nutrition 2000;7: 716-718.
28. Kasai H. Chemistry-based studies on oxidative DNA damage: Formation, repair, and mutagenesis. Free Radic Biol Med 2002;33: 450-456.
29. Wang Y, Hilton BA, Cui K, Zhu MY. Effects of antidepressants on DSP4/CPT-induced DNA damage response in neuroblastoma SH-SY5Y cells. Neurotoxicity Research 2015;28:154-170.
30. Andreazza AC, Frey BN, Erdtmann B, et al. DNA damage in bipolar disorder. Psychiatry Res 2007;153: 27-32.
31. Andreazza AC, Kauer-Sant'Anna M, Frey BN, et al. Effects of mood stabilizers on DNA damage in an animal model of mania. J Psychiatry Neurosci 2008;33:516-524.
32. Kropp S, Kern V, Lange K, et al. Oxidative stress during treatment with first-and second-generation antipsychotics. J Neuropsychiatry Clin Neurosci 2005;17: 227-231.
33. Czarny P, Kwiatkowski D, Toma M, et al. Impact of single nucleotide polymorphisms of base excision repair genes on DNA damage and efficiency of DNA repair in recurrent depression disorder. Mol Neurobiol 2017;54: 4150-4159.
34. Forlenza MJ, Miller GE. Increased serum levels of 8-hydroxy-2′-deoxyguanosine in clinical depression. Psychosom Med 2006;68: 1-7.
35. Talarowska M, Gałecki P, Maes M, et al. Malondialdehyde plasma concentration correlates with declarative and working memory in patients with recurrent depressive disorder. Mol Biol Rep 2012;39:5359-5366.
36. Licandro G, Khor HL, Beretta O, et al. The NLRP3 inflammasome affects DNA damage responses after oxidative and genotoxic stress in dendritic cells. Eur J Immunol 2013;43: 2126-2137.
37. Tang V, Wang J. Oxidative stress in bipolar disorder. Biochem Anal Biochem 2012; S2-002.
38. Wei YC, Zhou FL, He DL, et al. Oxidative stress in depressive patients with gastric adenocarcinoma. Int J Neuropsychopharmacol 2009;12:1089-1096.
39. Szebeni A, Szebeni K, DiPeri TP, et al. Elevated DNA oxidation and DNA repair enzyme expression in brain white matter in major depressive disorder. Int J Neuropsychopharmacol 2016;20:363-373.
40. Gałecki P, Talarowska M, Anderson G, et al. Mechanisms underlying neurocognitive dysfunctions in recurrent major depression. Med Sci Monit 2015; 21:1535- 1547.
41. Moylan S, Berk M, Dean OM, et al. Oxidative & nitrosative stress in depression: Why so much stress? Neurosci Biobehav Rev 2014;45: 46-62.
42. Ghadrdoost B, Vafaei AA, Rashidy-Pour A, et al. Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats. Eur J Pharm 2011;667: 222-229.
43. MacQueen GM, Campbell S, McEwen BS, et al. Course of illness, hippocampal function, and hippocampal volume in major depression. Proc Natl Acad Sci USA 2003;100:1387-1392.
44. Liu C S, Carvalho AF, McIntyre RS. Towards a "metabolic" subtype of major depressive disorder: shared pathophysiological mechanisms may contribute to cognitive dysfunction. CNS Neurol Disord Drug Targets 2014;13: 1693-1707.