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An evaluation of the differences in DNA damage in lymphocytes and repair efficiencies in patients with schizophrenia and schizoaffective disorder

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ABSTRACT

Schizophrenia and schizoaffective disorder are chronic and debilitating psychiatric disorders. The present study was designed to determine DNA damage in patients with schizophrenia and schizoaffective disorder to assess the roles of oxidative metabolism and DNA repair mechanisms in this process, to assess the contribution of drugs, and thus to demonstrate the differences between schizophrenia and schizoaffective disorder. Thirty schizophrenia and 30 schizoaffective disorder patients, each having at least five years of disease history, aged between 18 and 60 years with no physical or neurological diseases, and 30 healthy volunteers participated in the study. Psychometric scales were applied, and 5 ml of blood was taken from all participants. The DNA damage was measured in lymphocytes by the comet assay method; the total oxidative parameters by ELISA; OGG1 and NEIL1 gene expressions by real-time PCR; and the role of drugs by in vitro assays. The most important finding in this study was that patients with schizophrenia had significantly greater DNA damage than schizoaffective disorder patients and the controls. This study also provides evidence of high oxidative stress statuses and inadequate DNA repair capacities in patients with schizophrenia. Moreover, psychotropic drugs did not induce any DNA damage to the lymphocytes according to in vitro analyses. The use of clozapine and adequate repair processes of the patients were the decisive factors in the prevention of DNA damage. The results of this study provide a reexamination of schizoaffective disorder within the schizophrenia spectrum and indicate that schizoaffective disorder may be considered a different diagnostic category.

1. Introduction

Schizophrenia and schizoaffective disorder are chronic and disabling psychiatric disorders, the etiologies of which continue to be confounding and elusive (Soygur et al., 2007). Neurodegenerative and neurodevelopmental factors have been implicated in the etiologies of both conditions, but these disorders are frequently considered together in studies. As a result, very little is known about the etiological factors specific to schizoaffective disorder (Kaplan and Sadock, 2004; Malhi et al., 2008). One of the recent intriguing fields is genetics.

Genotoxicity, a term that describes mutations that cause structural changes in deoxyribonucleic acid (DNA) or that break the helix of DNA (Kent, 1998), may act as a predisposing factor for both schizophrenia and schizoaffective disorder. Reactive oxygen species cause oxidative DNA damage, react with cell membrane proteins, and prevent the intake of enzymes or neurotransmitters involved in natural processes (Dizdaroglu, 1999; Gergeroglu et al., 2007). The use of comet assays in clinical trials has shown increased DNA damage related to many diseases, such as breast cancer (Rajeswari et al., 2000) and Alzheimer's disease (Migliore et al., 2005), and contributed significantly to the research efforts to explain the pathogenic mechanisms of these diseases. DNA damage has also been revealed in psychiatric patient groups by the use of comet analyses. In the study by Andreazza et al. (2007), outpatients with bipolar disorder were shown to have more DNA damage than controls, and this damage was related to the severity of the disease symptoms. DNA damage has also been observed in schizophrenia patients (Muraleedharan et al., 2015; Psimadas et al., 2004). This knowledge revealed the need to study DNA damage processes.

Studies evaluating DNA damage have mostly focused on oxidative processes. In the evaluation of DNA damage, the repair mechanisms that cells can develop to repair such damage and maintain their genomic integrity must be considered, as well as the oxidative processes.

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8-OHG (8-hydroxyguanosine), one of the most important mutagenic lesions that cause oxidative damage, is a biomarker for oxidative DNA damage and is repaired by the enzyme OGG1 (8-oxoguanine DNA glycosylase) (Boiteux and Radicella, 1999). Polymorphisms in genes that are involved in DNA repair, including PARP1, OGG1, NEIL1, APE1, XRCC1, XRCC2, and XRCC3, can change protein function and activity and the DNA repair capacity of individuals. A lack of repair capacity is also a major cause of genetic instability (Ekmekci et al., 2008).

Another point that needs to be considered in the evaluation of DNA damage is the effects of drugs. According to the findings of Muraleedharan et al. (2015), the significant baseline DNA damage in schizophrenia, even prior to the initiation of antipsychotic treatment, is very important. Even so, the positive or negative influence of drugs on DNA damage cannot be disregarded and should be included in the evaluation.

The aims of this study are to determine and compare DNA damage in patients with schizophrenia and schizoaffective disorder, to assess the roles of oxidative metabolism and DNA repair mechanisms in this process, to assess the contribution of drugs, and thus to demonstrate the differences between schizophrenia and schizoaffective disorder and to re-examine schizoaffective disorder, which in the past was evaluated in the schizophrenia spectrum.

2. Method

The present study included 30 patients with schizophrenia and 30 patients with schizoaffective disorder, diagnosed according to the DSM V, at the Pamukkale University Medical School Department of Psychiatry. Each patient had at least five years of disease history, was aged between 18 and 60 years, and had no physical or neurological disease. The control group was composed of 30 healthy volunteers, matched for age and smoking habits, with normal mental capacities, no physical, neurological or psychiatric disease, and no medication.

Each participant or their legal representative was informed about the study, and written informed consent was obtained in accordance with the Declaration of Helsinki. Prior to the research, approval was received from Pamukkale University's Non-Interventional Clinical Investigations Ethics Committee dated 24.04.2015 and numbered 60116787-020/23917.

A sociodemographic data form was completed, and the SCID-5 (Structured Clinical İnterview for the DSM V) was administered to each participant (Kocabas et al., 2018). The PANSS (Positive and Negative Syndrome Scale) (Kostakoglu et al., 1999) and CGI (Clinical Global Impression) (Guy, 2000) were also administered to the patients with schizophrenia, and the PANSS, CGI, HAM-D (Hamilton Depression Rating Scale) (Akdemir et al., 2001) and YMRS (Young Mania Rating Scale) (Karadag et al., 2002) were administered to the patients with schizoaffective disorder. Patients were selected from among referral, walk-in clinic and inpatients to evaluate the relationship between the severity of the disease and DNA damage.

A total of 5 ml of venous blood was obtained from each participant for analysis. DNA damage was measured in lymphocytes via the comet assay method; TAS (total antioxidant status), TOS (total oxidant status) and OSI (oxidative stress index) measurements were obtained by ELISA; and OGG1 (8-oxoguanine DNA glycosylase) and NEIL1 (Nei-like DNA glycosylase) gene expression levels were obtained by real-time PCR. Additionally, the DNA-damaging effects of drugs commonly used by the patients were analyzed by the in vitro assay method of the blood samples obtained from the healthy volunteers in our study. Blood samples were analyzed immediately after being received and were studied in the dark so that the samples were minimally affected by environmental factors. Moreover, to minimize the effects of diet, fasting blood samples were obtained.

2.1. Comet assay

The comet assay was used to determine DNA damage and genotoxic situations of control in lymphocyte cells of patients with schizophrenia and patients with schizoaffective disorder. Lymphocytes were isolated by using histopaq (Histopaque, Sigma) with Leucosep™ Centrifuge Tubes. The cells were suspended in 0.1 M PBS (20,000 cells in 25 μl PBS). Three frosted slides per sample were prepared by adding three layers of low-melting point agarose gel (LMPA, 37 °C). The first layer consisted of 1.8% LMPA. A second layer of 25 μl of cell suspension and 175 μl of 1% LMPA was added to the solidified first layer. A third layer consisting of 1% LMPA (200 μl) was subsequently added. Once solidified, all slides were immersed in freshly prepared cold lysing solution [2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, 10 mM Tris (pH 10) and 10% DMSO] and incubated (1 h., 4 °C). Following incubation, the slides were placed in an electrophoresis buffer [0,3 M NaOH, 1 mM Na2EDTA; pH 13] for 20 min at 4 °C. The slides were then electrophoresed (25 V (300 mA, approx. 0.74 V/cm)) for 35 min at 4 °C. Then, the slides were washed three times with a neutralization buffer [0.4 M Tris; pH 7.5] for 5 min each. After this step, the slides were plunged into methanol for 5 min at −20 °C for fixation of the cells to the slides. After that, the slides were placed on a smooth surface and dried. Slides were stained with ethidium bromide (40 μl) prior to observation. Slides were viewed using a Nikon fluorescence microscope with 510–560 nm excitation and 590 nm emission filters. Images of at least 50 randomly selected comets on each triplicate slide were captured per sample at 20× magnification, and image analyses were performed using the comet assay IV Version 4.3.2 for Basler FireWire and are reported in μm.

Broken DNA molecules migrate at different rates in electrical fields, as they have different molecular weights and electric charges. The damaged pieces of DNA moving towards an anode present a comet-like image, but undamaged DNA cannot come out of the helical structure (Green et al., 1996; McKelvey-Martin et al., 1993). The parameters used to evaluate damage were the HL (head length, μm), TL (tail length, μm) HD (head density: percentage of DNA in the head), TD (tail density: percentage of DNA in the tail), and TMo (tail moment: expressed in μm, the value obtained by dividing the product of TL and TD by 100). As DNA damage increases, the head length increases, head density decreases; and the tail length, tail density, and tail moment increase (Fig. 1).

2.2. In vitro assay

An in vitro analysis was carried out to assess whether the drugs used by the patients could induce DNA damage. Valproic acid, olanzapine, clozapine, risperidone, quetiapine, paliperidone, amisulpride, and biperiden were the drugs most frequently used. In this scope, approximately 3 ml of blood was taken for each sample from three healthy volunteers selected from the control group, and one of these blood samples was used as a negative control without any drugs being present. Thus, DNA damage was evaluated in three different blood samples for reliability of the test results. The drugs were diluted in water at 0.1–0.5% of the therapeutic levels and incubated with peripheral blood (provided by healthy volunteers) for 30 min at 37 °C (Andreazza et al., 2007; Ucok and Soygur, 2010). After a period of incubation, DNA damage was evaluated using the comet procedure, as previously described. The selected doses were 1000 mg of valproic acid, 20 mg of olanzapine, 400 mg of clozapine, 6 mg of risperidone, 800 mg of quetiapine, 9 mg of paliperidone, 800 mg of amisulpride, and 4 mg of biperiden.

2.3. Measurement of TAS, TOS and OSİ

First, serum samples were obtained by centrifugation from blood samples taken from the control group and the patient groups. Samples were obtained with a Rel ASSAY Diagnostics Kit (Turkey). There were three chemicals in the kit, namely, buffer (reagent 1), prochromogen (reagent 2), and standard (reagent 3). Before starting the study, the

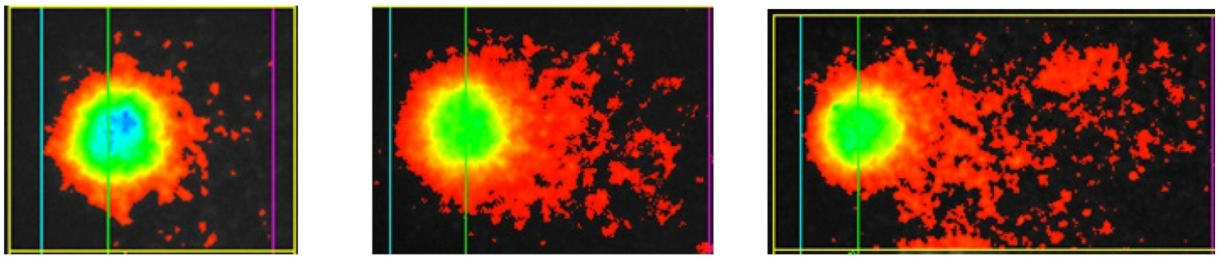

Fig. 1. *Comet* Images: Images of increasing DNA damage.

serum samples were treated with the chemicals in the kit according to the kit protocol. The samples were analyzed in duplicate and were read at the 660-nm wavelength with the aid of an Eliza reader (BioTek) and were calculated by a formula according to the manufacturer's instructions. Thereby, the TAS (total antioxidant status) and TOS (total oxidant status) values were measured.

OSI was calculated by the formula OSI = TOS/TAS* 1/10, according to the kit instructions.

2.4. RNA isolation and real-time PCR

Total RNA was isolated from the lymphocyte cells using TRIzol Reagent (İnvitrogen, USA) according to manufacturer's instructions. cDNA synthesis from the RNA template was performed via reverse transcription by using a Transcriptor First-Strand cDNA Synthesis Kit (Roche, Germany) according to the manufacturer's protocols. *OGG1* and *NEIL 1* gene expression analyses were performed by Step One Plus Real-Time RT-PCR (Applied Biosystems, USA) according to the SYBR Green qPCR Master Mix (Thermo Scientific, USA) protocol. The RT-PCR assay was performed using gene-specific primers. The expression results were normalized to the *beta-actin* gene (housekeeping gene) expression levels to calculate the relative expression ratios. The primer sequences used in this study are given in Table 1.

2.5. Statistical analysis

Statistical analyses were performed by using SPSS 17.0 for Windows. Continuous variables are shown as the mean, standard deviation, median, and interquartile ranges. Categorical variables are shown as the number and percentage. Comparisons of independent groups were conducted. Independent-samples *t*-tests and one-way ANOVA were used when the parametric test assumptions were satisfied, and when the parametric test assumptions were not satisfied, the Mann-Whitney *U* test and the Kruskal-Wallis analysis of variance were used. Chi-square analysis was used to compare categorical variables. Pearson correlational analyses were used to evaluate the relationships between continuous variables. The Bonferroni correction was performed to avoid type 1 errors, and the level of significance was determined by dividing the p-value (0.05) into the number of pairwise comparisons. Linear regression analyses (Backward) were performed to detect independent factors affecting DNA damage. The level of (p) 0.05 was used to determine statistical significance. All gene expression analyses of the findings were performed according to the delta-delta CT method and were quantitated with a computer program. The comparison of the groups was performed with "RT2 Profiles PCR Array data analysis," which is assessed statistically using the "Student *t*-test".

Table 1
Primer sequences of the genes used in this study.

	Name	Primer sequence
1	*Beta-actin*	F:TCCTCCTGAGCGCAAGTACTC R:CTGCTTGCTGATCCACATCTG
2	*OGG1*	F:CTGATGGCCCTAGACAAGCC R: ACTGAACAGCACCGCTTGG
3	*NEİL1*	F:GCAGTGGGAAGTCAGGTTCT R:GGCCTCATTCACAAACTGG

3. Results

The sociodemographic characteristics of the groups are presented in Table 2. There were no statistically significant differences between the groups in terms of age, gender, alcohol and smoking habits or body mass index (BMI). The patients with schizophrenia were found to have longer durations of illness and were living in rural areas (Table 2).

The results of the comet analysis were compared according to the head length (HL), tail length (TL), head density (HD), tail density (TD), and tail moment (TM) parameters, and the findings are presented in Table 3. The head lengths and tail lengths were found to be higher in the patients with schizophrenia than in the patients with schizoaffective disorder and control patients (Table 3). No significant differences were found in any of the parameters between the schizoaffective and control groups (Table 3).

The analysis of the relationships between the sociodemographic data and DNA damage showed that DNA damage was higher in the male group than in the female group (TL p = 0.004, TM p = 0.006). No significant relationship was observed between age and DNA damage (Table 4). Although there was an incremental increase in the extent of DNA damage in smokers, this change was not statistically significant (Table 4). However, there was a relationship between DNA damage and the number of cigarettes used; it was observed that DNA damage

Table 2
Sociodemographic characteristics of groups.

	Schizophrenia n (%)	Schizoaffective n (%)	Control n (%)	p
Age mean (ss)	39.43 ± 7.81	35.56 ± 9.78	36.66 ± 6.80	0.178[a]
Gender				
Female	9 (30.0)	16 (53.3)	17 (56.7)	0.079[b]
Male	21 (70.0)	14 (46.7)	13 (43.3)	
Cigarette				
Yes	16 (53.3)	17 (56.7)	14 (46.7)	0.745[b]
No	13 (43.3)	12 (40.0)	13 (43.3)	
Quit	1 (3.3)	1 (3.3)	3 (10.0)	
Alcohol				
No	28 (93.3)	26 (86.7)	21 (30.0)	0.060[b]
Rarely	2 (6.7)	4 (13.3)	6 (20.0)	
1–2 per week	0 (0.0)	0 (0.0)	3 (10.0)	
BMI mean (ss)	28.16 ± 4.46	27.09 ± 4.03	28.21 ± 5.46	0.685[c]
Duration of illness				
5–10 years	12 (40.0)	22 (73.3)	–	0.029[b]
10–20 years	14 (46.7)	7 (23.3)	–	
>20 years	4 (13.3)	1 (3.3)	–	
Living				
Urban	19 (63.3)	27 (90.0)	28 (93.3)	0.004[b]
Rural	11 (36.7)	3 (10.0)	2 (6.7)	
Working status				
Employed	7 (23.3)	8 (26.7)	28 (93.3)	0.000[b]
Unemployed	23 (76.7)	22 (73.3)	2 (6.7)	

[a] One Way ANOVA Test.
[b] Chi-square Test.
[c] Kruskal Wallis.

Table 3
Comparison of groups' comet values.

	Schizophrenia n (%)	Schizoaffective n(%)	Control n(%)	p[a]
Head length	84.12 ± 12.73	68.71 ± 17.73	72.16 ± 11.91	<**0.001**
Tail length	86.90 ± 32.58	67.51 ± 35.39	73.59 ± 22.73	**0.008**
Head density	77.13 ± 13.60	77.64 ± 12.12	78.13 ± 14.72	0.819
Tail density	22.86 ± 13.60	22.30 ± 12.11	21.77 ± 14.68	0.807
Tail moment	11.57 ± 10.65	9.90 ± 13.24	11.10 ± 9.28	0.407

[a] Kruskal Wallis Test (The difference in head length and tail length is due to the schizophrenia group.)

was high in patients who smoked more than one packet (20 cigarettes) a day (HL p = 0.044). There was no significant correlation between drinking alcohol and DNA damage. BMI was also not correlated with DNA damage (Table 4). The duration of disease was grouped as follows: five to 10 years, 10 to 20 years, and over 20 years. No significant correlation was found between the duration of disease and DNA damage (HL p = 0.436; TL p = 0.568; HD p = 0.315; TD p = 0.315; and TM p = 0.579). Furthermore, we found no relationship between the severity of the disease and DNA damage as a result of the correlational analysis performed with the applied psychometric scales (Table 4).

The medications of the groups are shown in Table 5. The usage rates of quetiapine, paliperidone and valproic acid were found to be low in patients with schizophrenia (Table 5). The relationship between comet values and the use of drugs such as quetiapine, olanzapine, risperidone, clozapine, paliperidone, amisulpride, valproic acid, and biperiden was also evaluated in the sample. We did not find any significant correlations between the use of drugs and head density, tail density or tail moment (p > 0.05). However, head lengths were found to be significantly lower in patients using paliperidone (p = 0.037) and in patients using clozapine (p = 0.017) than in the other patients. Similarly, in patients using valproic acid, both head length (p = 0.021) and tail length (p = 0.046) were significantly low. These results showed us that DNA damage is less severe in patients using clozapine, paliperidone, and valproic acid.

Table 4
Relationships between data and DNA damage.

		Head length	Tail length	Head density	Tail density	Tail moment
Age	r	0.143	0.076	0.196	0.197	0.189
	P[a]	0.179	0.478	0.065	0.062	0.075
Gender	P[b]	0.777	**0.004**	0.054	0.054	**0.006**
Smoke	P[c]	0.242	0.274	0.422	0.419	0.407
Alcohol	P[c]	0.393	0.296	0.204	0.235	0.252
BMI	r	0.181	0.133	0.022	0.019	0.040
	P[a]	0.087	0.210	0.841	0.858	0.711
PANSS	r	0.196	0.042	0.055	0.053	0.109
	P[a]	0.196	0.750	0.675	0.690	0.407
HAM-D	r	0.059	0.105	0.012	0.012	0.106
	P[a]	0.654	0.429	0.928	0.931	0.423
YMRS	r	0.089	0.053	0.007	0.008	0.156
	P[a]	0.504	0.688	0.956	0.955	0.237
CGI	r	0.178	0.122	0.171	0.170	0.152
	p[a]	0.174	0.353	0.191	0.194	0.247
TAS	r	0.009	0.015	0.119	0.121	0.059
	P[a]	0.930	0.889	0.266	0.258	0.580
TOS	r	0.059	0.093	0.135	0.134	0.118
	P[a]	0.580	0.383	0.204	0.209	0.268
OSI	r	0.047	0.082	0.114	0.113	0.092
	P[a]	0.662	0.444	0.283	0.288	0.391
OGG-1	r	−0.264	−0.258	0.027	0.027	0.124
	P[a]	**0.012**	**0.014**	0.798	0.799	0.244
NEIL-1	r	0.031	0.052	0.054	0.056	0.065
	P[a]	0.770	0.627	0.614	0.601	0.543

Bold face is the value that is statistically significant.
[a] Pearson Correlation Analysis.
[b] Mann Whitney Test.
[c] Kruskal Wallis Test.

Table 5
Medications of the groups.

	Schizophrenia % (n)	Schizoaffective % (n)	P[a]
Biperiden	30.0 (9)	26.7 (8)	1.000
Olanzapine	40.0 (12)	53.3 (16)	0.438
Amisulpirid	36.7 (11)	36.7 (11)	1.000
Risperidone	30.0 (9)	20.0 (6)	0.551
Valproic acid	10.0 (3)	66.7 (20)	<**0.001**
Quetiapine	30.0 (9)	63.3 (19)	**0.020**
Paliperidone	23.3 (7)	53.3 (16)	**0.034**
Clozapine	3,3 (1)	6.7 (2)	1.000

Bold face is the value that is statistically significant.
[a] Chi-square Test.

Measurements made through in vitro analyses before and after drug exposure showed that these medicines did not induce any DNA damage in the lymphocytes (Table 6).

Through analyses of the oxidative process in the schizoaffective disorder group, the TOS was measured as 2.96 (±3.22), and OSI was 0.26 (±0.32); in the schizophrenia group, the TOS was 6.95 (±5.34), and OSI was 0.48 (±0.49); and in the control group, the TOS was 9.73 (±1.94), and OSI was 0.95 (±0.44). The TOS and OSI values were found to be significantly lower in the patients with schizoaffective disorder than in the patients with schizophrenia (Table 7). In addition, the relationship between the oxidative processes and DNA damage was investigated by correlational analyses between comet values and the TAS, TOS and OSI values; and there was no significant relationship between DNA damage and the oxidative processes (Table 4).

OGG1 and NEIL 1 gene expression levels were examined in terms of DNA repair mechanisms. OGG1 gene expression fold changes were

Table 6
Comet values measured before and after in vitro drug exposure.

	Biperiden	Olanzapine	Amisulpirid	Risperidone
HLBI	67.77(±3.52)	67.77(±3.52)	67.77(±3.52)	67.77(±3.52)
HLAI	70.51(±4.47)	68.26(±2.22)	65.36(±2.11)	60.38(±3.68)
p[a]	0.109	1.00	0.285	0.109
TLBI	53.28(±9.60)	53.28(±9.60)	53.28(±9.60)	53.28(±9.60)
TLAI	59.75(±4.15)	60.57(±5.99)	51.87(±17.26)	56.95(±16.8)
p[a]	0.285	0.285	1.00	0.593
HDBI	85.84(±1.56)	85.84(±1.56)	85.84(±1.56)	85.84(±1.56)
HDAI	83.33	84.26(±5.59)	83,90(±8.63)	83.27(±8.53)
p[a]	0.285	0.593	0.593	1.00
TDBI	14.15(±1.56)	14.15(±1.56)	14.15(±1.56)	14.15(±1.56)
TDAI	16.66(±4.47)	15.73(±5.59)	16.09(±8.63)	16.72(±8.53)
p[a]	0.285	0.593	0.593	1.00
TMBI	5.50(±1.43)	5.50(±1.43)	5.50(±1.43)	5.50(±1.43)
TMAI	6.96(±1.81)	9.75(±6.00)	9.17(±8.03)	8.83(±7.28)
p[a]	0.109	0.285	0.593	0.593
	Valproicacid	Quetiapine	Paliperidone	Clozapine
HLBI	67.77(±3.52)	67.77(±3.52)	67.77(±3.52)	67.77(±3.52)
HLAI	66.67(±3.39)	70.6(±3.80)	71.80(±3.87)	74.78(±2.60)
p[a]	0.109	0.109	0.109	0.109
TLBI	53.28(±9.60)	53.28(±9.60)	53.28(±9.60)	53.28(±9.60)
TLAI	63.84(±12.68)	65.33(±16.6)	65.34(±0.46)	73.34(±4.60)
p[a]	0.109	0.285	0.109	0.109
HDBI	85.84(±1.56)	85.84(±1.56)	85.84(±1.56)	85.84(±1.56)
HDAI	80.43(±1.79)	81.36(±4.63)	82.89(±3.10)	83.90(±3.17)
p[a]	0.109	0.109	0.109	0.593
TDBI	14.15(±1.56)	14.15(±1.56)	14.15(±1.56)	14.15(±1.56)
TDAI	19.56(±1.79)	18.63(±4.63)	17.11(±3.10)	15.23(±4.04)
p[a]	0.109	0.109	0.109	1.00
TMBI	5.50(±1.43)	5.50(±1.43)	5.50(±1.43)	5.50(±1.43)
TMAI	7.58(±0.98)	9.35(±3.58)	9.49(±5.02)	6.83(±2.09)
p[a]	0.109	0.109	0.109	1.00

[a] Wilcoxon Paired Two Sample Tests HLBI(Head Length Before İncubation), HLAI(Head Length After İncubation), TLBI (Tail Length Before Incubation), TLAI(Tail Length After İncubation), HDBI(Head Density Before Incubation), HDAI(Head Density After Incubation) TDBI(Tail Density Before Incubation), TDAI(Tail Density After Incubation), TMBI (Tail Moment Before Incubation), TMAI (Tail Moment After Incubation).

Table 7
Comparison of oxidative metabolism values of patients with schizophrenia and schizoaffective disorder.

	Schizophrenia	Schizoaffective	p[a]
TOS	6.95 ± 5.34	2.96 ± 3.22	**0.001**
TAS	1.74 ± 0.47	1.59 ± 0.42	0.222
OSİ	0.48 ± 0.49	0.26 ± 0.32	**0.016**

Bold face is the value that is statistically significant.
[a] Mann Whitney Test TOS (Total oxidant status), TAS (total antioxidant status), OSI (Oxidative stress index).

measured as 27.02 (±3.70) in the schizophrenia group, 31.53 (±2.56) in the schizoaffective disorder group, and 27.20 (±2.90) in the control group. NEIL 1 gene expression fold changes were measured as 33.60 (±4.01) in the schizophrenia group, 34.77 (±2.17) in the schizoaffective disorder group, and 34.35 (±2.26) in the control group. OGG1 gene expression fold changes were significantly higher in the schizoaffective disorder group than in both the schizophrenia and control groups ($p < 0.001$) (Fig. 2). In addition, the relationship between DNA damage and repair mechanisms was investigated using correlational analyses between comet values and OGG1 and NEIL 1 gene expression levels. A negative correlation was found between OGG1 gene expression levels and DNA damage. There was no significant relationship with the NEIL1 values (Table 4).

Linear regression analysis (backward) was performed to detect independent factors affecting DNA damage, including the model age; gender; number of cigarettes smoked; BMI; level of OGG1; and use of paliperidone, valproic acid and clozapine. The use of clozapine was found to be an independent factor affecting head length ($B = 24.97$, standard error = 10.15, beta = 0.344, $p = 0.020$). OGG1 gene expression level was also found to be an independent factor affecting tail length ($B = -3.79$, standard error 1.81, beta = −0.352, $p = 0.044$).

4. Discussion

The most important finding in this study was that patients with schizophrenia had significantly greater DNA damage than the patients with schizoaffective disorder and the controls. This study also provided evidence of high oxidative stress statuses and inadequate DNA repair capacities in patients with schizophrenia. Moreover, psychotropic drugs did not induce any DNA damage in the lymphocytes.

Many studies have shown DNA damage in schizophrenia (Muraleedharan et al., 2015; Psimadas et al., 2004). Studies of oxidative metabolism have been previously performed in schizoaffective disorder patients (Bulbul et al., 2014). However, despite searching in the literature, we did not come across any study of DNA damage that used the comet assay method. Single-cell gel electrophoresis (comet assay), used also in humans, is a sensitive method for detecting major DNA damage (Tice et al., 2000). In our study, we found that the DNA damage in the schizophrenia group was higher than that in the schizoaffective disorder and control groups. To the best of our knowledge, our study is the first to evaluate DNA damage measured by the comet method with repair mechanisms, oxidative processes, and drug effects in schizophrenia and schizoaffective disorder patients.

The results of studies reporting the relationship between sex and genotoxicity have been contradictory (Moller et al., 2000; Muraleedharan et al., 2015). In our study, the detection of higher DNA damage in men than in women might be associated with the different smoking statuses of the male and female patients. Although there are conflicting studies, it is known that there is a relationship between aging and DNA damage (Jorgensen et al., 2013; Soares et al., 2014). We, however, did not find any correlation between age and DNA damage.

Studies have shown that free radicals in cigarettes induce DNA damage in smokers (Pryor et al., 1998) and effects on comet parameters (Moller et al., 2000). In our study, although the numbers showed more damage in smokers, we did not find any significant correlation between smoking and genotoxicity. In our interpretation, this finding was probably due to the small sample size since DNA damage was found to increase significantly as the number of cigarettes used increased.

Alcohol is known to cause oxidative DNA base modifications and breaks via the production of hydroxyacetyl free radicals during ethanol metabolism (Clot et al., 1994). There have been reports that this DNA damage is also related to frequent drinking (Weng et al., 2010). On the other hand, Horak et al. (2003) and Gaspari et al. (2003) claimed that there is no relationship between alcohol and DNA damage, and no relationship was found between alcohol use and DNA damage in this study. Additionally, DNA damage was not related to the duration of illness or the severity of symptoms, findings that were consistent with the literature (Andreazza et al., 2007; Muraleedharan et al., 2015).

Although there are conflicting results, studies have suggested that lower or higher BMIs are possibly associated with DNA damage (Dick et al., 2014; Mizoue et al., 2007). We found no relationship between BMI and DNA damage. However, eating problems and long-term changes in BMI are common both in schizophrenia and schizoaffective disorder due to disease characteristics and medications; therefore, longitudinal studies are needed.

As Muraleedharan et al. (2015) indicated, significant baseline DNA damage in schizophrenia is present even prior to the initiation of antipsychotic treatment. Additionally, intact genomic repair efficiency was noted in patients diagnosed with first episodes of schizophrenia, and it was observed that a longer duration of untreated illness decreased repair capacity. However, some previous studies have shown the influence of antipsychotics on DNA damage. For example, subchronic treatment of aripiprazole has been shown to cause DNA damage in the peripheral blood of rats (Picada et al., 2011). In another animal study,

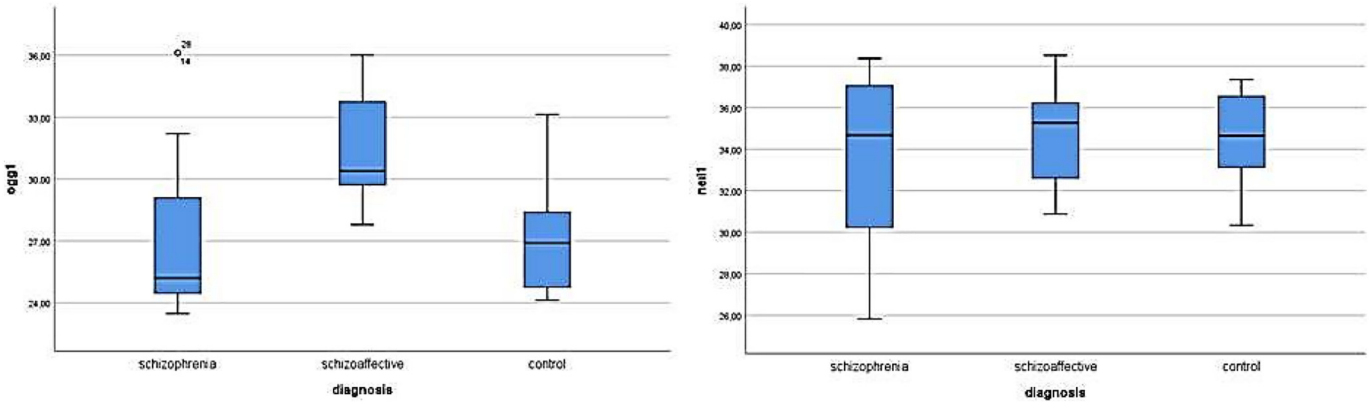

Fig. 2. OGG1 and NEIL1 gen expression levels of the groups.

Reinke et al. (2004) reported an increased level of lipid peroxidation product (thiobarbituric acid) with haloperidol but not with clozapine, and protein carbonyls increased with both haloperidol and clozapine. On the other hand, the researchers observed no increase in oxidative parameters with olanzapine. Parikh et al. (2003) found that chronic use of haloperidol causes oxidative stress and membrane lipid peroxidation but not olanzapine, risperidone, or clozapine use. In another in vitro study performed using lymphocyte cultures, olanzapine was found to cause damage only in high doses (Turkez and Togar, 2010). Psimadas et al. (2004) did not find any association of DNA damage or repair efficiency with Aloperidin, Nozinan, or Risperdal in human lymphocytes. Moreover, Tsai et al. (2013) found an incremental increase in antioxidant enzyme activity and glutathione peroxidase after four weeks of paliperidone treatment. Wakade et al. (2002) found an increase in bromodeoxyuridine, used to define newly divided cells, in rats that had been treated with atypical antipsychotics as distinct from typical antipsychotics; and demonstrated the stimulation of neurogenesis and neuronal repair with the use of atypical antipsychotics. All of these studies indicated to us that atypical antipsychotics cause less oxidative damage than typical antipsychotics. In our study, the use of quetiapine, olanzapine, risperidone, amisulpride, paliperidone, or clozapine did not increase DNA damage; in fact, DNA damage was significantly low in patients using clozapine and paliperidone, which was consistent with the literature. In the present study, the effects of drugs on DNA damage were also checked by the in vitro assay method, and consistent with the literature (Andreazza et al., 2007; Psimadas et al., 2004), our findings showed that these drugs did not induce any DNA damage to the lymphocytes.

The antioxidant and neuroprotective features of mood stabilizers have been demonstrated in animal and cell-based studies. For example, in animals with amphetamine-induced mania, it has been shown that lithium and valproate inhibit lipid peroxidation in the hippocampus and the prefrontal cortex (Frey et al., 2006). Similarly, lithium and valproate have been shown to prevent new DNA damage by modifying the oxidative balance (Andreazza et al., 2008). Furthermore, Tokarz et al. (2016) showed that, in chronic oxidative stress conditions, valproic acid reduces intracellular reactive oxygen species and damage to DNA. In another study, valproic acid was shown to enhance the antitumor effects of topoisomerase 2 inhibitors in mice with tumors (Marchion et al., 2005). Our results showed a significantly low level of DNA damage in patients using valproic acid, as found in the literature.

Compared to other tissues of the body, the central nervous system, with its high oxygen consumption and lipid-rich environment, is considered highly susceptible to oxidative stress and redox imbalances (Salim, 2014). Therefore, the fact that oxidative stress is implicated in several mental disorders, including schizophrenia, is not surprising. Albeit with conflicting results, oxidative stress is thought to play a role in the pathophysiology of schizophrenia (Dadheech et al., 2008; Wood et al., 2009). İnformation about the relationship between oxidative stress and schizoaffective disorder is limited in the literature. Bulbul et al. (2014) showed that oxidative stress was higher in patients with schizoaffective disorder than in patients with schizophrenia and bipolar disorder. However, in our study, the TOS and OSI values in the schizoaffective disorder group were significantly lower than those in the schizophrenia group. Oxidative stress levels are affected by many parameters, such as duration of illness, age, gender, smoking habits, nutrition habits, lifestyle, exercise, and BMI (Boskovic et al., 2011; Tuncel et al., 2015; Ustundag et al., 2006). Therefore, we regard this as a conflicting result. Moreover, in our study, we did not find any relationship between comet values and TAS, TOS or OSI values. This suggested that simply evaluating the oxidative processes in the formation of DNA damage would not be adequate, and more sensitive methods, such as comet assays, are required. In fact, in an animal study, Jorgensen (2013) showed a tendency for the genes involved in DNA repair to increase without an increase in urinary 8-oxo-7,8-dihydroguanosine levels, a product of nucleic acid oxidation and marker for chronic stress.

Polymorphisms in genes involved in DNA repair, such as OGG1 and NEIL1, can change the function and activity of proteins and the repair capacity of individuals. An inadequate repair capacity is an important contributing factor to genetic instability (Ekmekci et al., 2008). The OGG1 gene expression level and the use of clozapine were found to be independent factors in the present study. In other words, the use of clozapine and adequate repair processes of the patients were more decisive factors in the prevention of DNA damage.

Some limitations of the present study should be taken into consideration. It is known that patients with schizophrenia and schizoaffective disorder use multiple drugs during their illness. Less frequently used classes of drugs were not evaluated in the present study. Furthermore, limiting the drug selection to the last four weeks of treatment and the lack of scale to evaluate drug compliance are the other limitations of our study. Additionally, we cannot disregard the influence of nutritional habits, lifestyle, exercise, and other nonspecific factors on DNA damage. Thus, the lack of a mechanism for measuring such genotoxic variables is another limitation of this study. Further studies will help clarify this issue.

To conclude, the results of this study provide a reexamination of schizoaffective disorder within the schizophrenia spectrum and indicate that schizoaffective disorder may be differentiated from schizophrenia and may be considered a different diagnostic category.

Conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest.

Contributions

O.Z.T. conducted the literature review, conceptualized the study design, collected data, assisted with the analysis, wrote the first draft of the manuscript, and handled subsequent drafts after receiving coauthors feedback. O.O. designed the study and leads the project, conducted the literature review, commented on drafts and revised the manuscript. Y.D. and M.S. analyzed the bloods and assisted with the writing. All authors read and approved the final version of manuscript.

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