



Oxidatively-induced DNA damage and base excision repair in euthymic patients with bipolar disorder



Deniz Ceylan^{a,b,*}, Gamze Tuna^c, Güldal Kirkali^d, Zeliha Tunca^e, Güneş Can^f, Hidayet Ece Arat^g, Melis Kant^h, Miral Dizdaroglu^{i,**}, Ayşegül Özerdem^{b,e}

^a Vocational School of Health Services, Izmir University of Economics, Izmir, Turkey

^b Department of Neuroscience, Health Sciences Institute, Dokuz Eylul University, Izmir, Turkey

^c Department of Molecular Medicine, Health Sciences Institute, Dokuz Eylul University, Izmir, Turkey

^d Thoracic and Gastrointestinal Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Building 10, Bethesda, MD, 20892, USA

^e Department of Psychiatry, School of Medicine, Dokuz Eylul University, Izmir, Turkey

^f Department of Psychiatry, Mardin State Hospital, Mardin, Turkey

^g Department of Psychology, Istanbul Gelişim University, Istanbul, Turkey, Turkey

^h Department of Medical Biochemistry, Health Sciences Institute, Dokuz Eylul University, Izmir, Turkey

ⁱ Biomolecular Measurement Division, National Institute of Standards and Technology, Gaithersburg, MD, 20899, USA

ARTICLE INFO

Keywords:

Bipolar disorder
DNA damage
DNA repair
Base excision repair
Formamidopyrimidines

ABSTRACT

Oxidatively-induced DNA damage has previously been associated with bipolar disorder. More recently, impairments in DNA repair mechanisms have also been reported. We aimed to investigate oxidatively-induced DNA lesions and expression of DNA glycosylases involved in base excision repair in euthymic patients with bipolar disorder compared to healthy individuals. DNA base lesions including both base and nucleoside modifications were measured using gas chromatography-tandem mass spectrometry and liquid chromatography-tandem mass spectrometry with isotope-dilution in DNA samples isolated from leukocytes of euthymic patients with bipolar disorder ($n = 32$) and healthy individuals ($n = 51$). The expression of DNA repair enzymes OGG1 and NEIL1 were measured using quantitative real-time polymerase chain reaction. The levels of malondialdehyde were measured using high performance liquid chromatography. Seven DNA base lesions in DNA of leukocytes of patients and healthy individuals were identified and quantified. Three of them had significantly elevated levels in bipolar patients when compared to healthy individuals. No elevation of lipid peroxidation marker malondialdehyde was observed. The level of OGG1 expression was significantly reduced in bipolar patients compared to healthy individuals, whereas the two groups exhibited similar levels of NEIL1 expression. Our results suggest that oxidatively-induced DNA damage occurs and base excision repair capacity may be decreased in bipolar patients when compared to healthy individuals. Measurement of oxidatively-induced DNA base lesions and the expression of DNA repair enzymes may be of great importance for large scale basic research and clinical studies of bipolar disorder.

1. Introduction

Bipolar disorder (BD) is a chronic, severe and highly disabling psychiatric disorder, which is considered as one of the leading causes of disability amongst all medical and psychiatric conditions [1–3]. BD has previously been associated with increased mortality and morbidity due

to general medical conditions such as cardiovascular, metabolic or inflammatory diseases [4–12]. Despite vast uncertainties about the underlying molecular mechanisms, recent evidence has shown that increased oxidatively-induced DNA damage may have a central role in the pathophysiology of BD and increased cellular aging and comorbidity in BD [13–15]. Oxygen-derived free radicals are constantly generated as

Abbreviations: BD, bipolar disorder; HI, healthy individuals; FapyAde, 4,6-diamino-5-formamidopyrimidine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8-OH-Gua, 8-hydroxyguanine; 8-OH-dG, 8-hydroxy-2'-deoxyguanosine; 5-OH-5-MeHyd, 5-hydroxy-5-methylhydantoin; 5-OH-Cyt, 5-hydroxycytosine; ThyGly, thymine glycol; S-cdA, (5'S)-8,5'-cyclo-2'-deoxyadenosine; RT-PCR, real-time polymerase chain reaction; GC-MS/MS, gas chromatography/tandem mass spectrometry; LC-MS/MS, liquid chromatography/tandem mass spectrometry

* Corresponding author at: Vocational School of Health Services, Izmir University of Economics, Izmir, Turkey.

** Corresponding author.

E-mail addresses: denizceylan@gmail.com (D. Ceylan), miral.dizdar@nist.gov (M. Dizdaroglu).

<https://doi.org/10.1016/j.dnarep.2018.03.006>

Received 22 February 2018; Received in revised form 29 March 2018; Accepted 29 March 2018

Available online 30 March 2018

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by-products of aerobic metabolism. Oxidative stress occurs when enzymatic and non-enzymatic antioxidant defense systems are overwhelmed by elevated levels of oxygen-derived free radicals [16]. Oxidative stress damages biological molecules such as DNA, proteins and lipids, causing multiple forms of DNA damage including base and sugar modifications, strand breaks and DNA-protein cross-links [17]. Oxidatively-induced damage to DNA can initiate mutagenic processes and early aging [18]. This type of DNA damage has been shown to play a role in the pathophysiology of cardiovascular diseases, diabetes mellitus, various cancers and psychiatric disorders including BD [18–20]. Previous studies focusing on antioxidant enzymes and oxidatively-induced damage to proteins and lipids in BD reported consistent and significant alterations in antioxidant enzymes, lipid peroxidation and nitric oxide levels [21–23]. Increased levels of DNA single- or double-strand breaks have been shown in both postmortem brain tissues [24–26] and lymphocytes of patients with BD [27]. Moreover, levels of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) have been reported to be increased in blood [28,29] and urine samples of patients with BD [30,31]. Despite a plethora of known oxidatively-induced DNA base lesions, previous research in psychiatric disorders focused on 8-OH-dG only [20]. Therefore, there are no data on the alterations of the levels of DNA base lesions other than that of 8-OH-dG in BD.

Various DNA repair mechanisms exist to repair oxidatively-induced DNA base damage. The base excision repair (BER) is the major mechanism for the repair of this type of DNA damage. It recognizes and removes modified DNA bases by DNA glycosylases, followed by the activity of other enzymes to complete DNA repair [32–34]. In BER, OGG1 is a specific enzyme for the excision of 8-OH-Gua and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), whereas 4,6-diamino-5-formamidopyrimidine (FapyAde) and FapyGua are removed by NEIL1 and NEIL3, but not 8-OH-Gua [34]. Two studies showed that increases in expression of OGG1 were associated with depressive symptoms in cancer patients [35,36]. A decrease in BER capacity in recurrent depressive disorder [37], and down regulated OGG1 levels in rapid-cycling BD [38] have also been reported.

The objective of the present study was to investigate a more extensive set of markers of oxidatively-induced DNA damage and DNA repair enzymes in DNA samples isolated from leukocytes of euthymic patients with BP in comparison to healthy individuals.

2. Materials and methods

2.1. Participants

Patients with BD ($n = 32$) and healthy individuals ($n = 51$) were included in this study. The patients who had been euthymic for at least 6 months were recruited from the Bipolar Disorders Outpatient Unit, Department of Psychiatry, Dokuz Eylul University, Izmir, Turkey. Diagnoses were confirmed using the Structured Clinical Interview for the Diagnostic Manual of Mental Disorders [39] and clinical variables were recorded by experienced clinicians of the research team. Patients with neurological disorders, history of head trauma, chronic medical condition (e.g., hypertension, diabetes mellitus) and substance use were excluded. Other exclusion criteria included comorbid Axis I psychiatric diagnosis, neurodegenerative diseases, epilepsy or previous brain surgery, auditory or visual impairment, and being pregnant or breastfeeding. Symptomatic severity was assessed using Young Mania Rating Scale (YMRS) [40], Hamilton Depression Scale-17 (HAM-D) [41], Clinical Global Impression Scale (CGI) [42] and Global Assessment of Functionality (GAF) [43]. Healthy individuals with no known medical problems, no family history of major psychiatric or no neurological disorders, including dementia, mental retardation, cancer, cardiovascular disease or diabetes mellitus in the first-degree relatives or psychiatric history were enrolled in this study. Psychiatric conditions of the healthy individuals were confirmed by the Structural Clinical Interview for DSM-IV interview [38]. The study was approved by Dokuz Eylul

University Hospital Ethics Committee (Approval date: 12.07.2012; protocol no: 2012/16-13). All participants provided written informed consent.

2.2. Collection of the blood samples

Each participant provided 10 mL blood sample collected in EDTA-coagulated tubes (for leukocyte, RNA and plasma isolation) by venipuncture. At the day of the venipuncture, leukocytes were isolated from blood samples by density gradient separation using Histopaque-1119 and total RNA was extracted from 500 μ L blood samples using GeneJet RNA Purification Kit (Fermentas, MA, USA). Leukocytes were frozen at -80°C until DNA isolation. The RNA samples were frozen at -80°C until they were converted to first-strand cDNA with an oligo-2'-deoxythymidine (dT) 18 primer. The RNA samples were converted to first-strand cDNA using the First Strand cDNA Synthesis Kit (Fermentas, MA, USA) and were frozen at -80°C until quantitative real-time polymerase chain reaction (QRT-PCR) was performed.

2.3. DNA isolation and analysis

DNA was isolated from leukocytes by using salting-out/NaCl method [44]. DNA concentration was measured by recording the UV spectrum of each sample using an absorption spectrophotometer between the wavelengths of 200 nm and 350 nm. The absorbance at 260 nm was used to measure the DNA concentration. Subsequently, 50 μ g aliquots of DNA samples were dried in a SpeedVac under vacuum. According to a Material Transfer Agreement between Dokuz Eylul University, Izmir, Turkey and National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA, DNA samples were sent to NIST for analysis by gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2.4. Gas chromatography-tandem mass spectrometry

GC-MS/MS with isotope dilution was used to identify and quantify FapyAde, FapyGua, 8-OH-Gua, thymine glycol (ThyGly), 5-hydroxycytosine (5-OH-Cyt) and 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd). Aliquots (50 μ g) of DNA samples were supplemented with aliquots of internal standards FapyAde- ^{13}C , $^{15}\text{N}_2$, FapyGua- ^{13}C , $^{15}\text{N}_2$, 8-OH-Gua- $^{15}\text{N}_5$, ThyGly- $^2\text{H}_4$, 5-OH-Cyt- ^{13}C , $^{15}\text{N}_2$ and 5-OH-5-MeHyd- ^{13}C , $^{15}\text{N}_2$. DNA samples were dissolved in 50 μ L of an incubation buffer consisting of 50 mM phosphate buffer (pH 7.4), 100 mM KCl, 1 mM EDTA, and 0.1 mM dithiothreitol, and then incubated with 2 μ g of *E. coli* Fpg and 2 μ g of *E. coli* Nth for 1 h at 37°C to release DNA base lesions from DNA. Subsequently, 100 μ L ethanol were added to precipitate DNA. After centrifugation, supernatant fractions were separated, lyophilized and trimethylsilylated. Derivatized samples were analyzed by GC-MS/MS as described previously [45].

2.5. Liquid chromatography-tandem mass spectrometry

LC-MS/MS with isotope dilution was used to measure the levels of (5'S)-8,5'-cyclo-2'-deoxyadenosine (S-cdA) and 8-OH-dG, which is the 2'-deoxynucleoside form of 8-OH-Gua. Aliquots of S-cdA- $^{15}\text{N}_5$ and 8-OH-dG- $^{15}\text{N}_5$ as internal standards were added to an aliquot of 50 μ g of DNA samples, which were then dried in a SpeedVac. Subsequently, DNA samples were hydrolyzed with a mixture of nuclease P1, phosphodiesterase I and alkaline phosphatase according to a published procedure [45]. All samples were filtered using Millipore Microcon Ultracel YM-3 ultrafiltration membranes (Millipore, Bedford, MA) with molecular mass cutoff of 3 kDa by centrifugation at 12000xg for 30 min. LC-MS/MS analyses were performed using a Thermo-Scientific Finnigan TSQ Quantum Ultra AM triple quadrupole MS/MS system with an installed heated electrospray-ionization source, as described previously

Table 1
Demographic and clinical characteristics of the participants. Uncertainties are standard deviations.

	BD patients (n = 32)	Healthy individuals (n = 51)	p-value
Gender (number of females; their percentage) ^a	20; 62.5%	30; 58.8%	0.74
Age ^b	37.63 ± 9.96	36.28 ± 11.45	0.58
Smoking status (number of smokers; their percentage) ^a	15; 46.9%	16; 31.4%	0.16
Age of onset of illness ^b	26.83 ± 10.21		
Duration of illness (years) ^b	10.63 ± 8.72		
Total number of episodes ^b	5.63 ± 4.34		
Number of manic episodes ^b	3.20 ± 2.59		
Number of depressive episodes ^b	2.20 ± 2.46		
Clinical Global Impressions scale ^b	1.23 ± 0.43		
Global assessment of functionality ^b	86 ± 6.21		
Hamilton Depression Scale ^b	1.17 ± 1.49		
Young Mani Rating Scale ^b	0.47 ± 0.97		

^a Chi-Square.

^b independent samplest test.

[46]. Hydrolyzed DNA samples (20 µL injection volume, no waste mode) were analyzed using a Zorbax SB-Aq rapid resolution narrow-bore LC column (2.1 mm x 150 mm, 3.5 µm particle size) (Agilent Technologies, Wilmington, DE) with an attached Agilent Eclipse XDB-C8 guard column (2.1 mm × 12.5 mm, 5 µm particle size). In all instances, the autosampler and column temperature were kept at 5 °C and 40 °C, respectively. Mobile phases A and B were water and acetonitrile, respectively, both containing 0.1% formic acid (v/v). A gradient analysis of 3% (v/v) of B/min starting from 98% A/2% B (v/v) was used. After 6 min, B was increased to 60% in 0.1 min and kept at this level for 1 min and then another 13 min at 2% to equilibrate the column. The flow rate was 0.5 mL/min and the total analysis time was 20 min. Analysis by LC–MS/MS was performed using selected-reaction monitoring mode with the mass transitions m/z 284 → m/z 168 and m/z 289 → m/z 173 for 8-OH-dG and 8-OH-dG-¹⁵N₅, respectively, and with the mass transitions m/z 250 → m/z 164 and m/z 255 → m/z 169 for S-cdA and S-cdA-¹⁵N₅ respectively.

2.6. Measurement of the expression levels of DNA repair enzymes

The mRNA expression levels of human OGG1 and human NEIL1 were determined in samples of BD patients (n = 17) and healthy individuals (n = 19). Expressions of NEIL1 and OGG1 were measured by QRT-PCR using Maxima Sybr Green qPCR Master Mix (2x) (Fermentas, MA, USA) Kit. β-Actin was used as housekeeping gene. The amplification was performed in Light Cycler 1.5 (Roche Applied Science, Penzberg, Germany). Three independently prepared samples were used for each data point. The difference of cycle of threshold (Ct) between reference and target gene locus was observed by normalizing using housekeeping gene and calculating $\Delta\Delta Ct$ ratio ($\Delta\Delta Ct = \Delta Ct$ sample – ΔCt reference). Gene expression levels were calculated using the formula $2^{-\Delta\Delta Ct}$ [47,48].

2.7. Measurement of malondialdehyde

Malondialdehyde was extracted and analyzed according to a previously described method with slight modifications [49]. Briefly 40 µL plasma was diluted with 100 µL of H₂O and mixed with 20 µL of 2.8 mmol/L BHT in 95% ethanol, 40 µL of 81 g/L sodium dodecyl sulfate, and 600 µL of thiobarbituric acid (TBA) reagent consisting of 8 g/L TBA diluted 1:1 with 200 mL/L acetic acid adjusted to pH 3.5 with NaOH. The mixture was immediately incubated in a 90 °C water bath for 60 min and cooled on ice; 200 µL of H₂O and 1 mL of butanol-pyridine (15:1 by vol.) were then added. After vigorous mixing, the organic layer was separated by centrifugation (10 min at 10000 rpm). An aliquot (10 µL) was directly injected onto the high-performance liquid chromatography (HPLC). Calibration curves were constructed using 1,1,3,3-tetraethoxypropane (0.75 µmol/L–40 µmol/L). The separation

of the extracts was performed on an automated Shimadzu HPLC system (VP Series, Kyoto, Japan). The analytical column was a reverse phase silica based C18 column (GL Sciences/Inertsil ODS-3), with column dimensions of 150 × 4.6 mm, 5 µm. The mobile phase consisted of 70% 10 mM KH₂PO₄, pH 7.0 and 30% MeOH. The sample run was 5 min, with a flow rate of 0.8 mL/min, and fluorescence detection at 515 nm (excitation) and 553 nm (emission).

2.8. Statistical analyses

The IBM SPSS Statistics 23.0 (Chicago IL, USA) for Windows was used for data analysis. The Shapiro-Wilk's test was used to confirm normal distribution for continues variables. Where necessary, logarithmic transformations were applied in order to improve normality. Subsequently, transformed data were reassessed for normality. Group differences on continuous variables regarding demographic and clinical variables were evaluated with independent samples *t*-test. Chi-Square test was used to examine categorical data.

The statistical analysis of the GC–MS/MS and LC–MS/MS data was performed using the GraphPad Prism 7.01 software (La Jolla, CA, USA) and the unpaired, two-tailed nonparametric Mann Whitney test with Gaussian approximation and confidence level of 95%–99%. A *p*-value < 0.05 was assumed to correspond to statistically significant difference between medians.

3. Results and discussion

Sociodemographic and clinical characteristics of the BD patients and healthy individuals are described in Table 1. The groups did not differ from each other with regard to gender, age or smoking. One of the patients was drug-free, 9 patients were on mood-stabilizers as monotherapy (lithium or valproate), 19 patients were receiving a mood-stabilizer in combination with a second generation-antipsychotic, and one patient was receiving a mood-stabilizer in combination with an antidepressant. We identified and quantified six DNA base lesions by GC–MS/MS and two modified 2'-deoxynucleosides by LC–MS/MS in DNA samples from both BD patients and healthy individuals. The structures of these lesions are given in Fig. 1. It should be noted that 8-OH-dG is the 2'-deoxynucleoside form of 8-OH-Gua. Fig. 2A–H show the levels of the lesions shown in Fig. 1. A large group of samples was used for the measurements by GC–MS/MS. S-cdA and 8-OH-dG were measured in the remaining samples by LC–MS/MS. In various samples, some lesions could not be quantified with certainty. Therefore, the number of patient samples and that of healthy individual samples in the figures somewhat differ from lesion to lesion. The number of samples in each case is given in the legends of the figures. The levels of FapyAde, FapyGua and 5-OH-5-MeHyd in BD patients were significantly greater than those in healthy individuals. The confidence level was even 99%

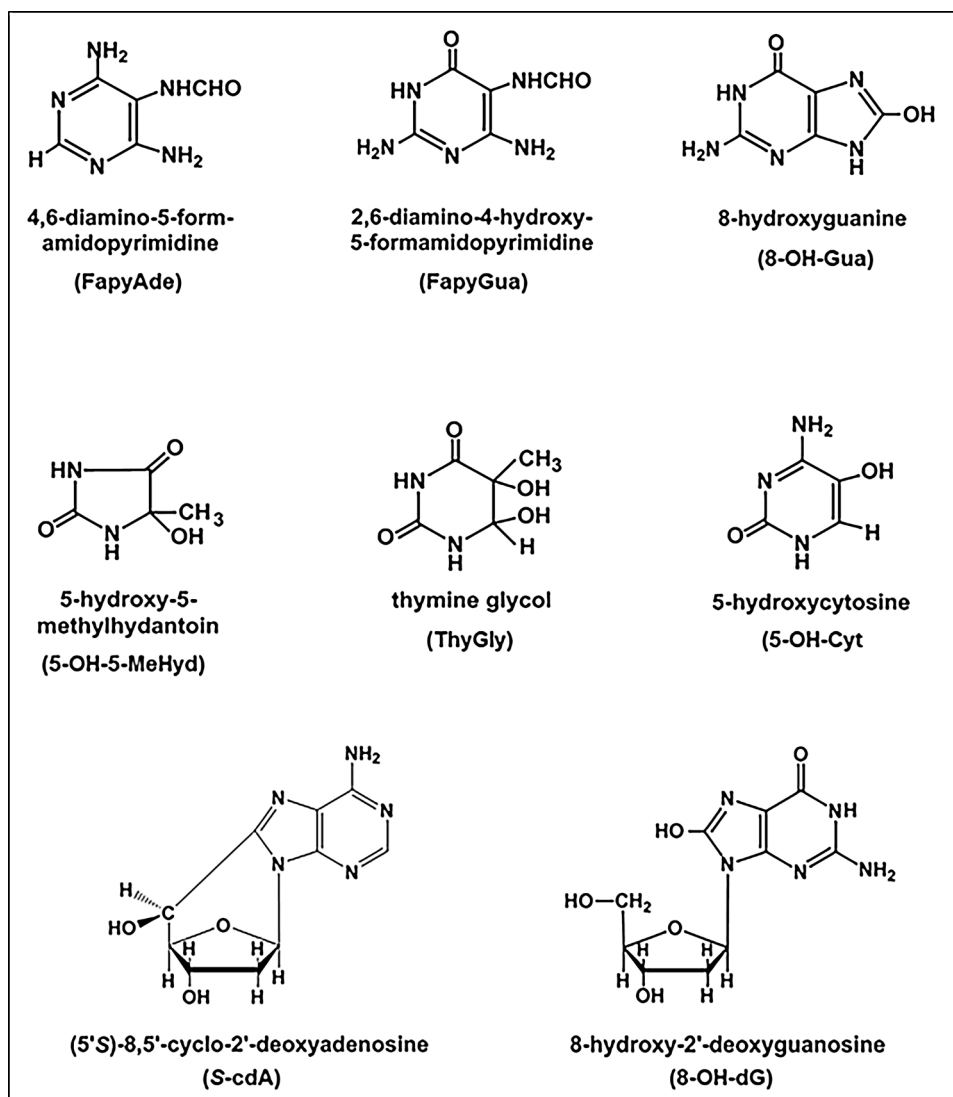


Fig. 1. The structures of the DNA lesions measured in this work.

for the latter two lesions. No significant difference between the levels of other lesions was observed when both groups were compared. Similarly, patient and healthy individual groups did not differ significantly with regard to the levels of malondialdehyde (Fig. 3). Fig. 4 illustrates the expression levels of OGG1 and NEIL1. The expression level of OGG1 in BD patients was 43% lower than that in healthy individuals and the difference between the two groups was significant after adjusting for age, gender and smoking ($F = 3.278$, $df = 4$, $p = 0.007$). On the other hand, no significant difference was observed between the expression levels of NEIL1 in both groups ($p = 0.49$) (Fig. 4).

Our results show that oxidatively-induced DNA damage occurs in DNA of BD patients compared to healthy individuals. Three out of the 8 DNA lesions measured in the present work exhibited significantly greater levels in BD patients than those in healthy individuals. To the best of our knowledge, this is the first study assessing different types of DNA lesions, representing oxidatively-induced damage to all four DNA bases in BD patients. The current knowledge on DNA base damage in BD has been based on the levels of 8-OH-dG only, which has been mostly measured as “the most prominent DNA base lesion” in biological samples because of the limitations of the methodologies used. Therefore, there were no available data on the lesions derived from adenine, cytosine and thymine, and one other important lesion of guanine, i.e., FapyGua. To this end, it is well known that hydroxyl radical attack on Gua produces both 8-OH-Gua and FapyGua by oxidation

and reduction of the same Gua-OH-adduct radical, respectively. Moreover, the yields of these products depend on the reaction conditions [50].

In the present work, 8-OH-Gua and 8-OH-dG were measured by two different techniques. It is important to note that GC-MS/MS and LC-MS/MS yielded almost identical levels of these compounds. In both cases, the levels 8-OH-Gua and 8-OH-dG did not differ between BD patients and healthy individuals. In contrast, the level of FapyGua was found to be significantly greater in BD patients than in healthy individuals ($p = 0.006$ with the confidence level of 99%). Thus, our results clearly show that the measurement of one DNA lesion such as 8-OH-dG (or 8-OH-Gua) only does not necessarily prove whether DNA damage in a given biological system occurs or not. Past published data on 8-OH-dG in BD patients differed among the studies. For example, a meta-analysis of the existing data [51] and more recent studies [29,30] showed greater levels of 8-OH-dG in BD patients than in healthy individuals, whereas several other studies reported unchanged levels of 8-OH-dG in both cases [28,30,52]. The discrepancy among these findings may be due to the methodological differences between the studies, and to the differences between clinical features of the study populations including illness state, course of illness, medications, smoking status, etc.

We also measured the expression levels of OGG1 and NEIL1 in both BD patients and healthy individuals. The expression level of OGG1 was

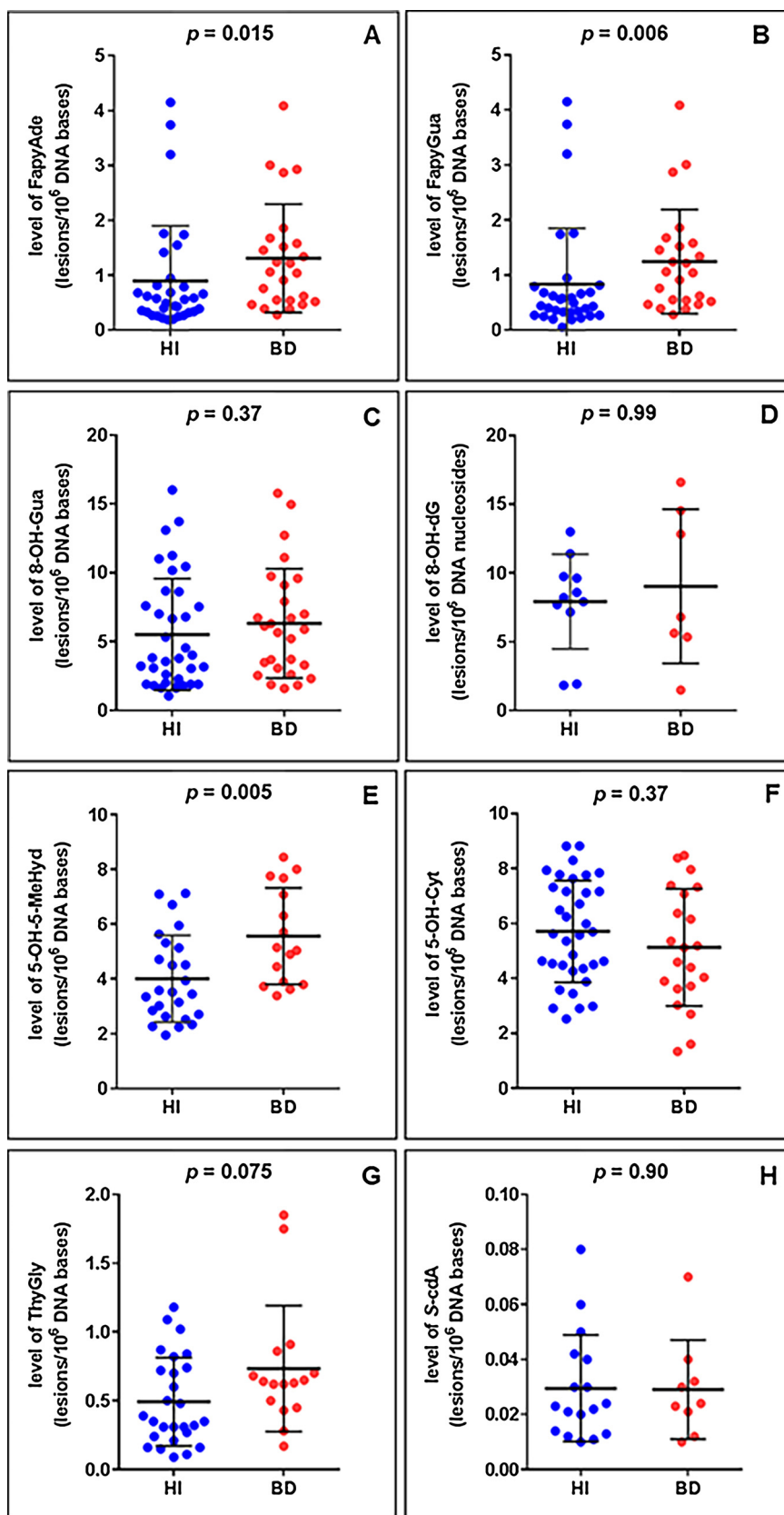


Fig. 2. The levels of DNA lesions. A: FapyAde in healthy individuals ($n = 33$) and BD patients ($n = 25$); B: FapyGua in healthy individuals ($n = 32$) and BD patients ($n = 24$); C: 8-OH-Gua in healthy individuals ($n = 36$) and BD patients ($n = 27$); D: 8-OH-dG in healthy individuals ($n = 11$) and BD patients ($n = 7$); E: 5-OH-5-MeHyd in healthy individuals ($n = 25$) and BD patients ($n = 16$); F: 5-OH-Cyt in healthy individuals ($n = 35$) and BD patients ($n = 21$); G: ThyGly in healthy individuals ($n = 28$) and BD patients ($n = 16$); H: S-cdA in healthy individuals ($n = 17$) and BD patients ($n = 9$). Uncertainties are standard deviations.

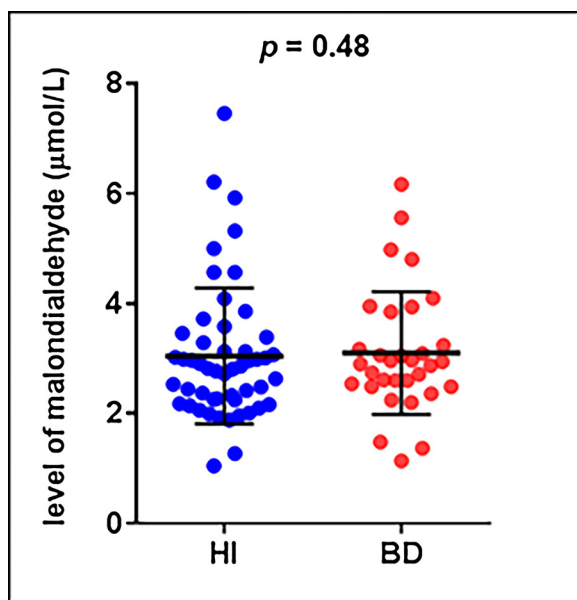


Fig. 3. The level of malondialdehyde in healthy individuals ($n = 51$) and BD patients ($n = 31$). Uncertainties are standard deviations.

found to be lower in BD patients than in healthy individuals. This is on a par with the previously reported down-regulated OGG1 expression in a rapid-cycling group of BD patients [38]. On the other hand, no elevation of NEIL1 expression was observed in BD patients. OGG1 and NEIL1 are bifunctional DNA glycosylases that are involved in the first step of the BER pathway to remove modified DNA bases from damaged DNA [32–34]. Their specificities differ from each other in that OGG1 removes FapyGua and 8-OH-Gua with similar excision kinetics, whereas NEIL1 is mainly specific for FapyAde and FapyGua, and to a lesser extent for 5-OH-5-MeHyd and ThyGly, but not for 8-OH-Gua [34]. In general, DNA glycosylases possess broad specificities for removal of DNA base lesions. For example, besides NEIL1, NTH1 and NEIL3 are specific for FapyAde removal in mammalian cells. The latter also removes FapyGua. NTH1 is the major DNA glycosylase that acts on ThyGly and 5-OH-Cyt in mammalian cells [33,34]. Therefore, the correlation of the levels of DNA base lesions with the expression levels of DNA glycosylases is quite complex, and not well understood. Low expression level of OGG1 in BD patients may be one of the factors leading to the greater level of FapyGua. On the other hand, similar levels of NEIL1 in both groups did not seem to affect the significant accumulation of FapyAde and 5-OH-5-MeHyd in BD patients. To this end, it is well known that various polymorphic variants of NEIL1 exist in human population such as NEIL1-Ser82Cys, NEIL1-Gly83Asp, NEIL1-Cys136Arg, NEIL1-Asp252Asn and NEIL1-Pro208Ser (reviewed in [33,34]). Among these variants, NEIL1-Gly83Asp and NEIL1-Cys136Arg have been shown to be completely devoid of glycosylase activity. Furthermore, NEIL1-Gly83Asp and NEIL1-Cys136Arg had significantly reduced activity. Such polymorphic variants may affect their binding, catalytic activity or protein–protein interaction with other DNA repair proteins such as PARP1, XRCC1 and CSB. These effects may cause the accumulation of typical substrates of NEIL1 such as FapyAde, FapyGua and 5-OH-5-MeHyd, as was found in this work. Future studies might include exomic sequencing of the NEIL1 gene to examine for such polymorphisms in BD patients.

The increased levels of oxidatively induced DNA lesions observed might be explained by increased oxidative stress in BD. Previous studies reported several alterations in oxidative markers including lipid peroxidation markers, antioxidant enzymes and nitric oxide levels in BD [53,54]. Malondialdehyde has been one of the most consistent lipid peroxidation marker that was found to be elevated in BD [55].

However, our results demonstrated no significant alterations in malondialdehyde levels, implying unchanged levels of oxidative stress load in the patient population compared to healthy individuals.

Several studies suggest that BD is associated with increased incidence for several medical comorbidities including cardiovascular, endocrine, inflammatory diseases [4–12], as well as various types of cancers [56–59]. DNA damage and reduced DNA repair capacity have been suggested to be one of the key mechanisms that underlie high clinical comorbidity, vulnerability to several cancers, neurocognitive decline and early aging in BD patients [13–15]. Some of the DNA base lesions identified in this work are strongly mutagenic and thus may contribute to those symptoms and others in BD patients. Thus, 8-OH-Gua and FapyGua pair with non-cognate Ade and lead to G→T transversion mutations [60–64]. The level of 8-OH-Gua was not increased in BD patients; however, 8-OH-Gua is readily oxidized, leading to the formation of spiroiminohydantoin (Sp) and 5-guanidinohydantoin (Gh), which exhibit mutagenic effects as well as cytotoxic effects [65]. Sp and Gh were not measured in the present work. Facile oxidation of 8-OH-Gua may prevent its accurate measurement *in vivo*. FapyAde leads to A→T transversions and is mutagenic, albeit to a lesser extent than FapyGua [66]. 5-OH-5-MeHyd can be a lethal or mutagenic lesion, because it constitutes a replication block for some DNA polymerases or is by-passed by low fidelity polymerases [67–70] (for more information on the mutagenic effects of oxidatively-induced DNA base lesions identified in this work, see reviews [33] and [71]).

Clinical characteristics of the patient population of this study needs consideration while interpreting our results. It is important to note that our study population consisted of only euthymic patients with BD. Previously, manic or depressive patients were shown to have higher levels of 8-OH-dG lesions than euthymic patients [28,31]. Further studies are needed to identify different types of DNA lesions presenting oxidatively-induced damage to all DNA bases measured in this study and DNA repair enzyme profiles across different states of BD (mania, depression and euthymia). Smoking status of participants might affect the levels of DNA damage/repair [72,73]. In this work, however, double comparisons between smokers and non-smokers did not show any significant difference with respect to DNA lesions and DNA glycosylases (Table 2).

Medication effect is the other parameter that requires attention while studying DNA damage in any patient population. Our patient population was predominantly on mood stabilizing medications (i.e., lithium or valproate). Previous evidence suggested that lithium and valproate treatments may have antioxidant properties [74–79], and may decrease DNA damage [24,74–79]. However, some studies showed similarly higher 8-OH-dG levels in both unmedicated [29] and medicated patient populations [27,28,30,31], leaving the effect of psychotropic medications on DNA damage equivocal. Future prospective studies specifically designed to understand the effects of mood stabilizing treatments on DNA damage/repair processes are needed.

In conclusion, our results show enhanced levels of several oxidatively-induced DNA base lesions and reduced levels of OGG1 in leukocytes of patients with BD when compared with healthy individuals. These findings suggest a defect in base excision repair in BD. Measurement of oxidatively-induced DNA base lesions and expression levels of DNA repair enzymes may be of great importance for large scale basic research and clinical studies of BD, contributing to a comprehensive understanding of the DNA damage/repair mechanisms in BD.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

DNA samples were transferred to NIST from Dokuz Eylul University and analyzed at NIST pursuant to a Material Transfer Agreement

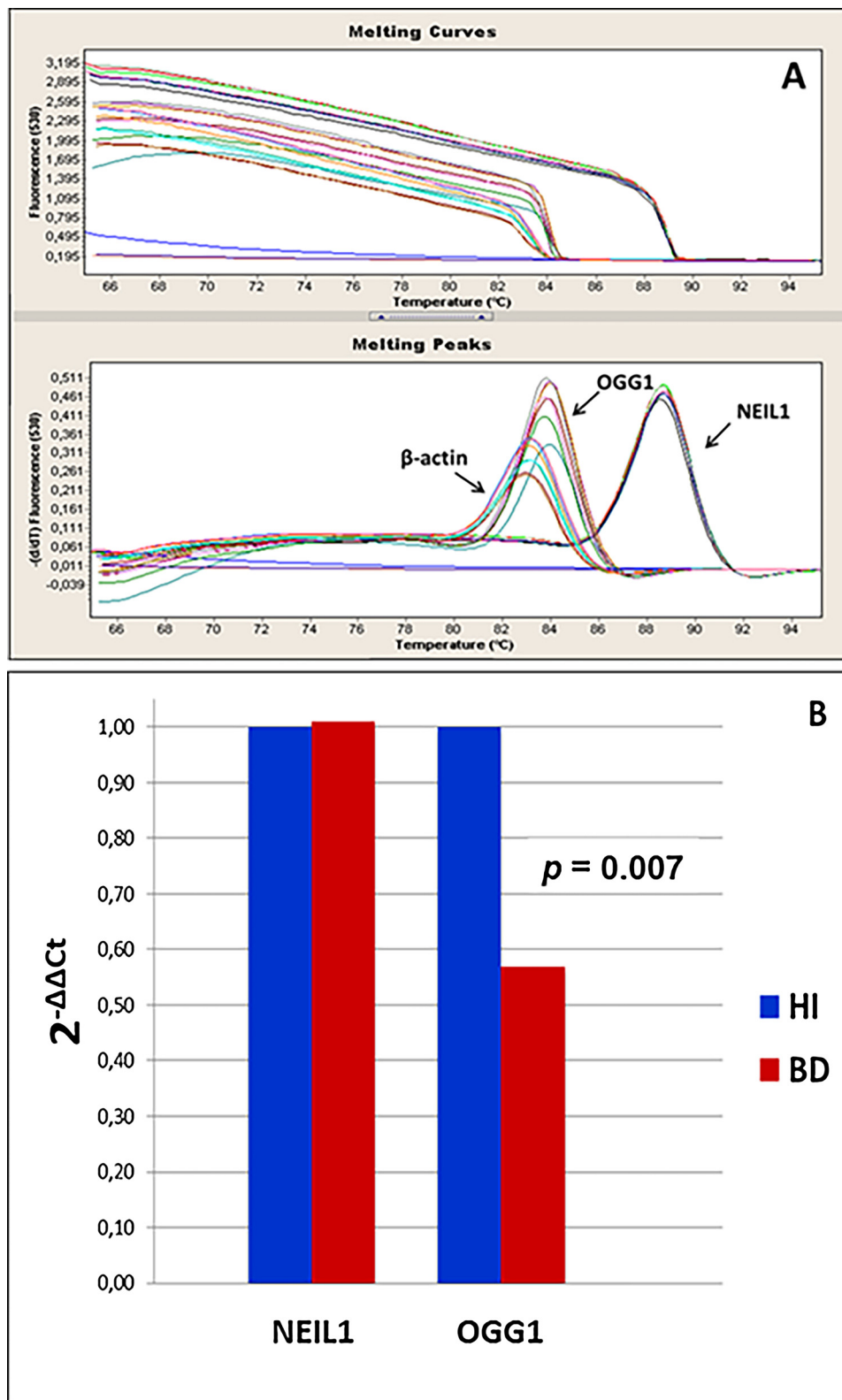


Fig. 4. A: Analysis of the melting curves of the OGG1, NEIL1 and β -actin (housekeeping) genes: The coefficient of variations (% CV) for the expressions of the genes NEIL1, OGG1 and β -actin were 0.6, 1.2 and 1.5, respectively. B: Expression profiles of OGG1 and NEIL1: OGG1 ($2^{-\Delta\Delta C_t}$) 0.43 (\downarrow) ($p = 0.007$) and NEIL1 ($2^{-\Delta\Delta C_t}$) 0.01 (\uparrow) ($p = 0.489$). The results are adjusted by age, gender, smoking status using linear regression. $2^{-\Delta\Delta C_t}$ (fold change) is computed using $\alpha = 0.016$. Ct: cycle of threshold; ΔC_t : the difference of Ct between target gene and β -actin (housekeeping) gene.

Table 2

Comparisons of markers between smokers and non-smokers. Uncertainties are standard deviations.

	Non-smoker participants (n = 31)	Smoker participants (n = 52)	p-value ^a
DNA lesion (lesions/10⁶ DNA bases)			
FapyAde	1.06 ± 1.11	1.35 ± 1.26	0.249
FapyGua	0.90 ± 0.80	1.22 ± 1.10	0.234
8-OH-Gua	6.30 ± 4.44	5.15 ± 3.12	0.484
8-OH-dG	8.38 ± 3.77	8.32 ± 5.59	1.000
5-OH-5-MeHyd	4.38 ± 1.75	5.27 ± 1.94	0.140
5-OH-Cyt	5.25 ± 2.03	5.85 ± 1.84	0.360
ThyGly	0.57 ± 0.39	0.67 ± 0.47	0.788
S-cdA	0.03 ± 0.02	0.03 ± 0.02	0.357
Malondialdehyde (μmol/L)	3.06 ± 1.25	3.08 ± 1.11	0.843
DNA glycosylases			
OGG1 (ΔCt)	6.31 ± 0.83	6.46 ± 0.89	0.566
NEIL1 (ΔCt)	2.76 ± 0.47	2.77 ± 0.51	0.986

Ct: cycle of threshold; ΔCt: the difference of Ct between target gene and β-actin (housekeeping) gene.

^a Mann-Whitney U test.

between NIST and Dokuz Eylul University. Certain commercial equipment or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose. The Psychiatric Association of Turkey awarded an Encouragement Prize to this research project.

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