Higher DNA and RNA damage from oxidative stress in patients with newly diagnosed bipolar disorder and their unaffected first-degree relatives

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Introduction

Bipolar disorder (BD) is a highly heritable[1], potentially progressive disorder with an increasing risk of manic and depressive episodes at decreasing intervals over time[2,3] and further associated with disability, cognitive impairment, decreased quality of life as well as a reduced life expectancy of 8-12 years[4]. The pathophysiology is still unclear, however, dysmetabolism[5] and cardiovascular diseases[6] are twice as common in patients with BD compared with the general population, adding to the reduced life expectancy in these patients[6–9]. Further, somatic disease occurs at significantly younger ages[6,9] in patients with BD compared with the general population in line with the theory of accelerated aging[10]. Oxidative stress is recognized as a major trigger for cardiovascular disease[11] and may possibly represent a common pathophysiological mechanism shared by dysmetabolic conditions, cardiovascular diseases and BD.

It has been hypothesized that cumulative inflammation and oxidative stress contribute to the pathophysiology of BD through increased generation of reactive oxidative species and DNA/RNA nucleoside damage causing telomere shortening and alterations in the electron transport chain in the mitochondria[12,13]. A validated method of determining systemic effects of oxidative damage is the measurement of the DNA damage marker 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and RNA damage marker 8-oxo-7,8-dihydroguanosine (8-oxoGuo) in urine by ultraperformance liquid chromatography with tandem mass spectrometry[14,15]. Nevertheless, *in vivo* studies of urine 8-oxoGuo and 8-oxodG levels are limited. A meta-analysis of oxidative stress, including 117 patients with BD and 113 healthy control individuals (HC) found higher oxidative stress measured as DNA/RNA damage in blood in patients with BD[16] in line with three subsequent studies from our group [17–19]. We have found increased urinary RNA and DNA oxidative stress markers in patients with rapid cycling BD[19] and in patients hospitalized for acute mania[17] compared with HC, as well as in cerebrospinal fluid of patients with BD compared HC[18].

Since it is largely unknown whether oxidative stress levels are increased in patients with newly diagnosed BD and in their unaffected first-degree relatives (UR) the aim of this study was to compare

the urinary 8-oxoGuo and 8-oxodG levels in patients with newly diagnosed/first-episode BD, their UR, and HC without personal or first-degree history of affective disorders.

Methods and Materials

Study design

The present study is a cross-sectional investigation of baseline data from the ongoing longitudinal Bipolar Illness Onset Study (BIO), which aims to identify composite biomarkers for BD in patients newly diagnosed with BD, their UR and HC. A full research protocol has been published for the BIO cohort study [20].

The study protocol has been approved by the Committee on Health Research Ethics of the Capital Region of Denmark (protocol No. H-7-2014-007) and the Danish Data Protection Agency, Capital Region of Copenhagen (RHP-2015-023). The study complies with the Declaration of Helsinki and its ethical principles.

Participants

Patients were recruited from the Copenhagen Affective Disorder Clinic, Copenhagen. Inclusion criteria were an ICD-10 diagnosis of BD or a single manic or hypomanic episode. Their first-degree relatives (i.e. siblings and children) were invited to participate in the study, if they did not have an ICD-10 diagnosis below F34.0. Age- and sex-matched healthy individuals without a personal or a first-degree family history of psychiatric disorders that had required treatment, were recruited from Danish Blood Bank at Rigshospitalet, Copenhagen. Additional clarification is described in the research protocol[20].

Diagnostics, data collection and clinical assessment

An initial diagnosis was made by medical doctors specialized in psychiatry according to the ICD-10 and DSM-IV criteria for type I and type II BD. After informed consent, medicine or psychology Ph.D. students verified the diagnosis using the Schedules for Clinical Assessment in Neuropsychiatry

(SCAN)[21] categorizing patients into BD type I or type II. Clinical assessments of severity of depressive and manic symptoms were done using the Hamilton Depression Scale-17 items (HAM-D17)[22] and the Young Mania Rating Scale (YMRS)[23]. Medication usage was noted, the past months quality of sleep was assessed using the Pittsburgh Sleep Quality Index (PSQI)[24] and activity level during the previous week was assessed using the International Physical Activity Questionnaire (IPAQ)[25]; moreover, BMI, educational level, daily alcohol intake and smoking habits were recorded. Lightly dressed and without shoes, height and weight were measured.

Laboratory methods

Urine collection and preparation

Urine samples were collected between 07.30 and 10.00 AM. A freshly voided spot urine sample was obtained using a standard sampling kit without any additives (In Vitro, Fredensborg, Denmark). The sample was kept on ice and centrifuged at 4°C and 1590*g* for 15 min, after which aliquots of 1.5 ml were transferred to Eppendorf tubes and stored at −80 °C until analysis.

Urinary 8-oxodG and 8-oxoGuo levels

Urine samples were collected between June 2015 to April 2020 and analyzed in the Laboratory of Clinical Pharmacology, Rigshospitalet, Copenhagen, Denmark and analyzed using the same standardized procedures as in previous studies[17]. Creatinine concentrations were measured in the urine samples for oxidative stress levels to be divided by creatinine levels in accordance with Jaffe's reaction[15].

Statistical analyses

Descriptive data were analyzed by chi-squared test for categorical data and by Kruskal-Wallis test for continuous data. Further mixed effect regression models were applied for continuous data accounting for familial relationship between relatives as a random effect. Continuous data were

presented as median and interquartile range when nonparametric and categorical data were presented as number and percentage.

First, we compared 8-oxoGuo and 8-oxodG levels, respectively, in unadjusted mixed effect regression models, with familial relationship as random effect to account for the correlation between family-related individuals. Second, for our main analyses, we compared 8-oxoGuo and 8-oxodG levels in mixed effect regression models adjusted for age and sex as independent variables. We repeated these models adjusted for age and sex, exclusively including patients in full or partial remission, defined as a score <14 on the HAM-D17 and <14 on the YMRS, their UR and HC to investigate 8-oxoGuo and 8-oxodG levels as potential trait factors. Finally, we employed a fully adjusted model with sex, age, BMI, alcohol units per week, current smoking status (yes/no), HAMD-17, YMRS, PSQI (total score) and IPAQ (total score).

In multiple regression analyses among patients, we explored the association between 8-oxoGuo and 8-oxodG levels and medication and illness related variables. In these models, illness duration, current psychotropic medication in the form of antidepressants (yes/no), antiepileptics (yes/no), antipsychotics (yes/no) and lithium (yes/no) were entered as predictors along with age, sex, BMI, alcohol units per week, smoking (yes/no), HAMD-17 (total score), YMRS (total score), PSQI (total score) and IPAQ (total score) as covariates. Further, in similar models the four categorical psychotropic medication groups were substituted with the categorical variable receiving psychotropic medication (yes/no) and finally, illness duration was exchanged with number of affective episodes.

In post hoc analyses we compared 8-oxoGuo and 8-oxodG levels between the three groups (BD, UR and HC) in current non-smokers in models adjusted for sex and age. We also examined patients with BD type I and II, respectively, in the models with UR and HC adjusted for sex and age. Subsequently, we examined 8-oxoGuo and 8-oxodG levels in patients with less than two years of untreated BD, UR and HC in models adjusted for sex and age.

The natural logarithm was applied to 8-oxodG and 8-oxoGuo if assumptions of normal distribution were not met. Results were presented as back transformed values with a parameter estimate, B, expressing the ration between increments in independent variables. All model assumptions were met. SPSS version 25 was used (SPSS for Windows Inc., Chicago, IL). The level of significance was set at $p < 0.05$.

Results

Demographic and clinical characteristics

We included 360 patients with newly diagnosed BD, 92 UR and 197 HC. Demographic and clinical characteristics of the study participants are presented in Table 1. The three study groups showed no significant difference in sex or age distribution, except for UR being statistically significantly younger, see Table 1.

Nucleoside damage from oxidative stress in patients with bipolar disorder, their unaffected first-degree relatives and healthy control persons.

In unadjusted models the level of 8-oxoGuo was increased 17.1% in patients with BD compared with HC (BD vs. HC: B=1.171, 95%CI=1.124-1.220, p<0.001) and 11.5% in UR compared with HC (UR vs. HC: B=1.115, 95%CI=1.052-1.182, p<0.001), see Figure 1. No significant difference was found between patients with BD and UR (BD vs. UR, B=1.050, 95%CI=0.996-1.107, p=0.07). 8-oxodG levels were 21.3% higher in patients with BD (B=1.213, 95%CI=1.146-1.284, p<0.001) and 25.9% higher in UR (B=1.259, 95%CI=1.161-1.366, p<0.001) compared with HC, see Figure 2. Patients with BD and UR had similar levels of 8-oxodG (B=0.963, 95%CI=0.895-1.037, p=0.3).

In our main analyses adjusted for sex and age, 8-oxoGuo was 17.1% higher in patients with BD (p<0.001) and 13.3% higher in UR (p<0.001) compared with HC, see Table 2, Model 1. Levels of 8-oxodG was 21.2% higher in patients with BD (p<0.001) and 26.6% higher in UR (p<0.001) compared with HC, see Table 2, Model 1. When considering only patients in full or partial remission, 8-oxoGuo was 17.2% higher for patients with BD compared with HC (B=1.172, 95%CI=1.122-1.225, p<0.001)

and levels of 8-oxodG in patients with BD was 20.2% higher than HC (B=1.202, 95%CI=1.131-1.276, p<0.001), whereas levels did not differ between patients with BD and UR (p=0.2).

In the fully adjusted models adjusted for sex, age, BMI, alcohol, smoking, HAMD-17, YMRS, IPAQ and PSQI: 8-oxoGuo was 15.5% higher in patients with BD (p<0.001) and 13.4% higher in UR (p<0.001) compared with HC, see Table 2, Model 2. Levels of 8-oxodG was 11.7% higher in patients with BD (p=0.009) and 20.9% higher in UR (p<0.001) compared with HC, see Table 2, Model 2.

Associations between illness duration, medication and oxidative stress levels in newly diagnosed patients with bipolar disorder

In analyses within patients, Table 3, lithium treatment was associated with 9.1% higher levels of 8-oxoGuo (B=1.091, 95%CI=1.026-1.60, p=0.006) and 15.8% higher levels of 8-oxodG (B=1.158, 95%CI=1.062-1.262, p=0.001), whereas antidepressants were associated with 10% lower levels of 8-oxodG (B=0.900, 95%CI=0.813-0.996, p=0.042).

Post hoc explorative analyses

Sensitivity analyses exclusively comparing current non-smokers revealed higher levels of RNA and DNA oxidative stress markers in patients with BD compared with HC (B=1.15-1.13, p<0.001). In models adjusted for sex and age, levels of 8-oxoGuo (p=0.6) and 8-oxodG (p=0.4) did not differ between patients with BD type I and type II. Finally, exclusively including patients with a duration of BD <2 years did not alter main findings.

Discussion

The study profited from assessing reliable and validated measurements of systemic oxidative stress levels, 8-oxoGuo and 8-oxodG, in a large well characterized study population with a total of 649 participants comprising 360 patients with newly diagnosed BD, 92 of their UR and 197 HC. The levels of 8-oxoGuo and 8-oxodG were statistically significantly higher in both patients with BD and UR compared with HC in our main analyses and further withstood adjustment for several possible

predictors in fully adjusted analyses. To account for the possible state variation of oxidative stress, sub-analyses compared patients with BD in full or partial remission and found similarly higher 8-oxoGuo and 8-oxodG levels compared with HC. These findings were consistent both in BD type I and II.

Interpretation of findings in patients with BD

Our findings of increased levels of both the DNA and RNA oxidation marker in newly diagnosed patients with BD replicates previous findings from our group[17,19]. In accordance with the two prior studies from our group[17,19], we did not find an association between illness duration or number of affective episodes and levels of oxidative stress.

Despite a median illness of ten years and median delay in diagnosis of four years, 97% of our patients were diagnosed within the last two years. Analyses exclusively including those with less than two years of untreated BD did not alter main findings, supporting a key role of oxidative stress in early stages of BD.

Increased oxidative stress occurs across a number of both somatic and psychiatric illnesses, such as diabetes, cancer, schizophrenia and unipolar disorder[26,27] and is associated with early cardiovascular disease[28], thereby not specific to BD, but rather suggests a shared pathophysiology of these diseases. Despite the putative antioxidant effect of lithium[29], we found that treatment with lithium was associated with higher levels of oxidative stress and, similarly to Munkholm et al., antidepressants were associated with lower levels[19].

Interpretation of findings in UR

Contrasting prior findings from our group[30], we found higher 8-oxoGuo and 8-oxodG in UR compared with HC, suggesting that oxidative stress might already be present prior to onset of BD. Our results comport with the finding of shorter telomere lengths in both patients with BD and their UR in a small study by Vasconcelos-Moreno[13]. Interestingly, we have previously assessed the 30-year cardiovascular risk on a subsample of the included patients with BD, UR and HC and found a higher

risk in both patients with BD and UR compared with HC[31], possibly explained by the higher levels of oxidative stress in line with a recent prospective study where high 8-oxoGuo levels were associated with cardiovascular mortality in patients with type 2 diabetes[32].

In line with findings of low heritability and high degree of environmental influence on DNA and RNA nucleoside damage[33], our findings could reflect underlying epigenetic alterations in patients with BD and UR growing up in potentially stressful environments in families having more mental illness and with a risk of exposure to childhood trauma, smoking, drug abuse and psychological and social stress[28,34].

Possible predictors

In line with obesity being associated with increased levels of oxidative stress[35], we found increasing BMI to be associated with higher levels of 8-oxoGuo in analyses within patients with BD. However, against expectations, we found decreasing BMI to be associated with higher 8-oxodG. Prior studies have associated male sex with higher oxidative DNA damage in plasma[36] and in urine[37] corresponding with our findings within patients with BD. Unexpectively, we found significantly higher oxidative stress levels in female sex compared with male sex in our analyses comparing the three groups. As expected[38], smoking was also associated with elevated oxidative stress levels. Sensitivity analyses of non-smokers revealed similarly higher oxidative stress levels in patients with BD and UR compared with HC supporting an illness effect. Furthermore, sleep plays a putative bidirectional role in oxidative stress levels[39], whilst physical exercise seems to increase oxidative stress[19], but neither PSQI total score nor IPAQ total score was associated with 8-oxoGuo or 8-oxodG.

Strengths and limitations

It is a strength that we included a large cohort of well-described patients with newly diagnosed BD, their UR and HC. A high degree of standardization was applied with urinary samples being collected in a fasting state between 7.30-10 A.M. on the same day as participants were having a thorough clinical evaluation.

However, some limitations apply to our study. First, our control group was recruited among blood donors without a personal or first-degree family history of psychiatric illness adding to the fact, that blood donors represent a super healthy population[40]. Nonetheless, the recruited blood donors were from the same catchment area as our patients with BD and matched with patients on sex and age. More patients with BD and UR were active smokers than HC. Nevertheless, results were still highly significant when adjusting for smoking as well as when excluding smokers. Our findings regarding alcohol intake should be interpreted with caution, as cessation was strongly recommended to the patients upon admission in clinic. Alltogether, we consider our control group a pragmatic choice, as other ways to recruit HC such as via advertisements or national registers result in low response rates and likely selection bias.

Moreover, we used dichotomous treatment categories, which fail to capture the effects of dose and duration of treatment or overlap of treatments. Thus, our results regarding psychotropic medication should be interpreted with caution. Finally, we did not adjust for oral contraception, which has been shown to elevate 8-oxoGuo and 8-oxodG in female sex [17,19] and could explain our unexpected finding of higher DNA and RNA damage in female sex compared with male sex in our analyses between the three groups.

In conclusion, we found higher levels of systemic nucleoside damage in patients with newly diagnosed BD and their UR compared with HC. Our findings underline the presence of oxidative stress in UR and early stages of BD. These findings show that elevated 8-oxoGuo and 8-oxodG may represent trait markers of BD and could play an etiological role in the development of BD.

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Table 1. Demographic variables and oxidative stress levels in patients with newly diagnosed bipolar disorder (BD), their unaffected relatives (UR) and healthy controls persons (HC)

	BD	UR	HC	P-value
N	360	92	197	
Age (years)	29.0 [24.2-36.9]	26.7 [22.8-32.1]	27.6 [24.3-36.1]	0.946^{BD-HC} 0.011^{UR-HC} 0.003^{BD-UR}
Sex (% female)	234 (65.0)	54 (58.7)	127 (64.5)	0.991^{BD-HC} 0.608^{UR-HC} 0.501^{BD-UR}
Education (years total)	15 [13-17]	15 [13-17]	16 [15-17]	$<0.001^{BD-HC}$ 0.008^{UR-HC} 0.408^{BD-UR}
BMI (kg/m^2)	24.5 [22-27]	23.4 [21-27]	23.7 [22-26]	0.015^{BD-HC} 0.666^{UR-HC} 0.019^{BD-UR}
Number of smokers (%)	156 (43.9)	19 (21.1)	22 (11.2)	$<0.001^{BD-HC}$ 0.174^{UR-HC} $<0.001^{BD-UR}$
Alcohol (units per week)	2 [0-7]	2 [1-6]	5 [2-10]	0.006^{BD-HC} 0.023^{UR-HC} 0.704^{BD-UR}
HAMD-17	9 [5-15]	2 [0-4]	0 [0-2]	$<0.001^{BD-HC}$ 0.002^{UR-HC} $<0.001^{BD-UR}$
YMRS	3 [0-7]	0 [0-2]	0 [0-1]	$<0.001^{BD-HC}$ 0.735^{UR-HC} $<0.001^{BD-UR}$
IPAQ	1983 [1040-3685]	2400 [997-4845]	2798 [1538-4262]	0.167^{BD-HC} 0.989^{UR-HC} 0.287^{BD-UR}
PSQI	8 [6-11]	5 [3-7]	4 [3-5]	$<0.001^{BD-HC}$ 0.015^{UR-HC} $<0.001^{BD-UR}$
BD I	112 (31.1)	-	-	-
BD II	248 (68.9)			
Age of onset (years)	17 [14-21]	-	-	-
*Illness duration (years)	10 [6-16]	-	-	-
**Untreated bipolar disorder (years)	4 [1-10]	-	-	-
Affective episodes	12.5 [6-27]	-	-	-
Current affective state				
Remission	211 (58.9)	-	-	-
Mild/moderate depressive episode	86 (24)	-	-	-

Severe depressive episode	8 (2.3)	-	-	-
Manic episode	1 (0.3)			
Hypomanic episode	30 (8.4)	-	-	-
Mixed episode	20 (5.6)	-	-	-
N/A	2 (0.6)	-	-	-
Current psychotropic medication				
No psychotropic medication	61 (16.9)	-	-	-
Antidepressant treatment	47 (13.1)	-	-	-
Antipsychotic treatment	120 (33.3)	-	-	-
Antiepileptic treatment	187 (51.9)			
Lithium treatment	110 (30.6)	-	-	-

Continuous variables are presented as median [interquartile range]. Categorical variables are presented as n (%). Abbreviations: BMI: Body Mass Index; HAM-D-17: Hamilton Depression Rating Scale; YMRS: Young Mania Rating Scale; IPAQ: International Physical Activity Questionnaires; PSQI: Pittsburgh Sleep Quality Index; N/A: Not applicable.

*Illness duration was defined as time from first episode (i.e. depressive, manic, hypomanic or mixed episode).

**Untreated bipolar disorder was defined as time from first manic, hypomanic or mixed episode to time of diagnosis.

Table 2. Levels of oxidative stress markers 8-oxoGuo and 8-oxodG in patients with newly diagnosed bipolar disorder (BD), their unaffected first-degree relatives (UR) and healthy control persons (HC)

Model		B	95%CI	P-value
1	**8-oxoGuo**			
	BD vs. HC	1.171	1.125-1.219	<0.001
	UR vs. HC	1.133	1.069-1.200	<0.001
	BD vs. UR	1.034	0.981-1.090	0.216
	Age	1.004	1.002-1.006	<0.001
	Male vs. female sex	0.949	0.913-0.985	0.007
	8-oxodG			
	BD vs. HC	1.212	1.145-1.283	<0.001
	UR vs. HC	1.266	1.167-1.374	<0.001
	BD vs. UR	0.957	0.889-1.031	0.248
	Age	1.000	0.998-1.003	0.801
	Male vs. female sex	0.920	0.872-0.971	0.002
2	**8-oxoGuo**			
	BD vs. HC	1.155	1.089-1.226	<0.001
	UR vs. HC	1.134	1.067-1.206	<0.001
	BD vs. UR	1.019	0.955-1.087	0.578
	Age	1.004	1.002-1.006	<0.001
	Male vs. female sex	0.947	0.909-0.986	0.009
	BMI	1.004	0.999-1.008	0.091
	Alcohol	0.996	0.993-0.999	0.020
	Smoking	1.040	0.996-1.087	0.078
	HAMD-17	1.000	0.997-1.004	0.835
	YMRS	0.999	0.994-1.004	0.753
	IPAQ	0.998	0.992-1.004	0.518
	PSQI	1.000	1.0000-1.000	0.983
	8-oxodG			
	BD vs. HC	1.117	1.029-1.214	0.009
	UR vs. HC	1.209	1.110-1.317	<0.001
	BD vs. UR	0.924	0.845-1.010	0.083
	Age	1.002	0.999-1.005	0.162
	Male vs. female sex	0.941	0.889-0.996	0.037
	BMI	0.990	0.983-0.996	0.001
	Alcohol	0.998	0.994-1.003	0.416
	Smoking	1.136	1.069-1.208	<0.001
	HAMD-17	1.007	1.001-1.012	0.015
	YMRS	0.997	0.989-1.004	0.353
	IPAQ	0.999	0.990-1.007	0.765
	PSQI	1.000	1.000-1.000	0.779

Model 1 adjusted for age and sex; Model 2 adjusted for age, sex, BMI, alcohol, smoking, HAM-D17, YMRS, IPAQ and PSQI. Abbreviations: BMI: Body Mass Index; HAMD-17: Hamilton Depression Rating Scale; YMRS: Young Mania Rating Scale; IPAQ: International Physical Activity Questionnaires; PSQI: Pittsburgh Sleep Quality Index.

Table 3. Oxidative stress markers 8-oxoGuo and 8-oxodG in patients with newly diagnosed bipolar disorder

Model		B	95%CI	P-value
1	**8-oxoGuo**			
	Age	1.003	1.000-1.007	0.082
	Male vs. female	1.071	1.014-1.132	0.015
	BMI	1.006	1.001-1.012	0.030
	Alcohol	0.996	0.993-1.000	0.070
	Smoking	1.057	1.004-1.112	0.034
	HAMD-17	0.993	0.939-1.050	0.809
	YMRS	1.013	0.908-1.130	0.820
	IPAQ	1.000	1.000-1.100	0.178
	PSQI	0.994	0.987-1001	0.082
	Illness duration	1.001	0.997-1.005	0.667
	Affective episodes*	1.000	1.000-1.001	0.411
	Antidepressants	1.021	0.949-1.098	0.574
	Antipsychotics	1.020	0.966-1.077	0.470
	Antiepileptics	0.992	0.940-1.048	0.776
	Lithium	1.091	1.026-1.160	0.006
	Receiving medicine**	0.978	0.914-1.046	0.513
	8-oxodG			
	Age	1.001	0.995-1.006	0.805
	Male vs. female	1.107	1.024-1.196	0.011
	BMI	0.989	0.981-0.997	0.005
	Alcohol	1.001	0.996-1.006	0.710
	Smoking	1.177	1.096-1.264	<0.001
	HAMD-17	0.937	0.866-1.013	0.103
	YMRS	0.976	0.837-1.137	0.751
	IPAQ	1.000	1.000-1.000	0.072
	PSQI	0.996	0.986-1.005	0.381
	Illness duration	1.001	0.995-1.006	0.831
	Affective episodes*	1.000	0.999-1.001	0.767
	Antidepressants	0.900	0.813-0.996	0.042
	Antipsychotics	0.983	0.911-1.060	0.649
	Antiepileptics	1.028	0.953-1.109	0.477
	Lithium	1.158	1.062-1.262	0.001
	Receiving medicine**	0.983	0.894-1.082	0.730

Model 1 adjusted for age, sex, BMI, alcohol, smoking, HAMD-17, YMRS, IPAQ and PSQI. Abbreviations: BMI: Body Mass Index; HAMD17: Hamilton Depression Rating Scale; YMRS: Young Mania Rating Scale; IPAQ: International Physical Activity Questionnaires; PSQI: Pittsburgh Sleep Quality Index.

*In a second model illness duration was substituted with affective episodes.

**In a third model the four categorical psychotropic medication variables were substituted with the categorical variable “Medication vs. medication free”.

Figure 1

Boxplot of oxidative stress marker 8-oxoGuo levels (nM/mM) in newly diagnosed patients with bipolar disorder (BD), their unaffected first-degree relatives (UR) and healthy control persons (HC). The lower and upper hinges represent the first and third quartiles. The upper and lower whiskers extend from the hinge to the largest and lower value, respectively. Data beyond the end of the whiskers are plotted individually.

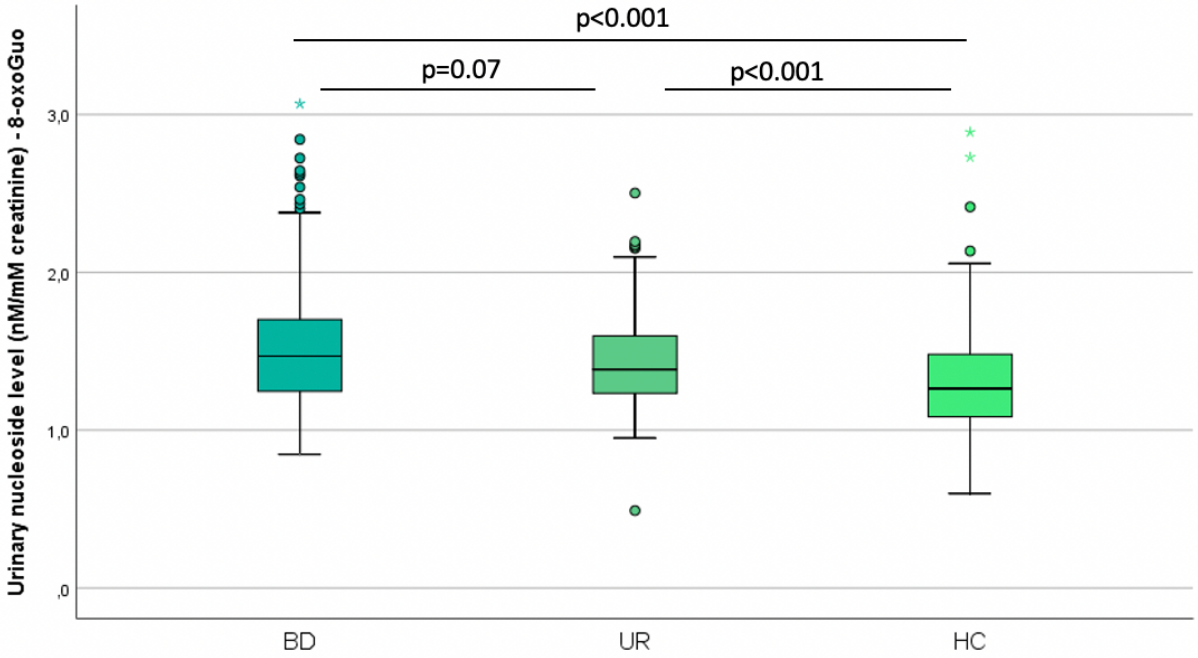

Figure 2

Boxplot of oxidative stress marker 8-oxodG levels (nM/mM) in newly diagnosed patients with bipolar disorder (BD), their unaffected first-degree relatives (UR) and healthy control persons (HC). The lower and upper hinges represent the first and third quartiles. The upper and lower whiskers extend from the hinge to the largest and lower value, respectively. Data beyond the end of the whiskers are plotted individually.