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Research report

Systemic oxidatively generated DNA/RNA damage in clinical depression: Associations to symptom severity and response to electroconvulsive therapy

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ABSTRACT

Background: Depression has been associated with increased oxidative stress and hypothesized to accelerate aging. Nucleic acid damage from oxidation is a critical part of the aging process, and a suggested early event in age-related somatic morbidities that are also prevalent in depression, such as dementia and type 2 diabetes. We hypothesized that increased severity of depression is associated with increased systemic oxidatively generated DNA and RNA damage, and that this increase is attenuated by an effective antidepressant treatment.

Methods: The urinary excretion of markers of systemic oxidatively generated DNA and RNA damage, 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo), respectively, were determined in healthy controls ($N=28$), moderately depressed, non-medicated patients ($N=26$) and severely depressed patients eligible for electroconvulsive therapy (ECT) ($N=29$). In the severely depressed patient group, samples were also obtained 1 week after the completion of ECT.

Results: Systemic RNA damage from oxidation, as measured by 8-oxoGuo excretion, was higher with increasing severity of depression (controls < moderately depressed < severely depressed) (P for trend $=0.004$). The 8-oxoGuo excretion was further increased after clinically effective ECT compared with pre-ECT values ($P=0.006$). There were no differences in 8-oxodG excretion between the groups or pre- vs. post-ECT.

Limitations: Small sample size and the inclusion of both unipolar and bipolar patients in the severely depressed group.

Conclusions: Severe depression is associated with increased systemic oxidatively generated RNA damage, which may be an additional factor underlying the somatic morbidity and neurodegenerative features associated with depression. Due to the lack of normalization by clinically effective ECT, the phenomenon does not appear to be causally linked to the depressive state per se.

1. Introduction

The depressed state has been proposed to accelerate aging (Wolkowitz et al., 2010). Depression is associated with an increased telomere attrition (Simon et al., 2006) and risk of age-related disorders such as dementia (Saczynski et al., 2010), both of which appear to be positively correlated to the number of depressive episodes (Kessing, 2012; Kessing and Andersen, 2004; Wolkowitz et al., 2011). Correspondingly, depression is linked to increased natural-cause mortality (Mykletun et al., 2009; Wulsin et al., 2005).

Oxidative stress on the genome is recognized as a central mediator of cellular aging (Finkel and Holbrook, 2000; Sahin and DePinho, 2010) and a key regulator of telomere length (Richter and von Zglinicki, 2007). Oxidative stress on telomeric DNA impacts on telomere stability and function, yielding the cell susceptible to apoptosis or senescence (Opresko et al., 2005; Wang et al., 2010; Zhang et al., 2007). Furthermore, DNA and RNA damage from oxidation has been implicated as an early event in Alzheimer's disease (Lovell and Markesbery, 2007; Lovell et al., 2011; Nunomura et al., 2009; Tanaka et al., 2007) as well as in other age-related medical disorders which are prevalent in

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depression, such as type 2 diabetes (Broedbaek et al., 2011; Mezuk et al., 2008).

A large body of evidence supports a role for oxidative stress in depression, including increased peripheral markers of oxidative damage to lipids, proteins and DNA in depressed individuals, as well as a reduced plasma antioxidants, antioxidant enzyme function and total antioxidant capacity (recently reviewed by (Maes et al., 2011)). In a recent post-mortem study of gene expression profiles in the frontal cortex, major depressive disorder was associated with molecular signs of inflammation, apoptosis and oxidative stress (Shelton et al., 2011). Furthermore, some studies suggest that conventional antidepressants and mood stabilizers may act in part through antioxidant mechanisms (Berk et al., 2011; Maes et al., 2011), and conversely that antioxidants have antidepressant properties (Berk et al., 2008; Scapagnini et al., 2012). Hence, oxidative stress has both been hypothesized to constitute a pathogenetic mechanism in depression (Ng et al., 2008), to be a mediator of the telomere erosion and somatic morbidity associated with depression (Wolkowitz et al., 2010), and to be a biological phenomenon underlying the signs of structural and functional brain abnormalities associated with the cumulative exposure to depression (Moylan et al., 2012).

With specific regard to oxidatively generated modifications of DNA in depression, cross-sectional human studies have found increased serum levels of 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG), a marker of DNA damage from oxidation, in clinical depression (Forlenza and Miller, 2006), and a positive association between depressive symptoms and intra-DNA levels of 8-oxodG in peripheral leucocytes in a non-clinical population (Irie et al., 2003), as well as in clinically depressed individuals (Irie et al., 2005). In another study, urinary 8-oxodG was increased in depressed individuals with comorbid myalgic encephalomyelitis/chronic fatigue syndrome (Maes et al., 2009). However, a recent report did not find any significant associations between urinary 8-oxodG and depressive symptoms in a large, non-clinical population of Japanese office workers ($N=511$) (Yi et al., 2012).

Urinary 8-oxodG and its ribonucleoside analogue 8-oxo-7,8-dihydroguanosine (8-oxoGuo) are specific and well-characterized markers of whole-body oxidatively generated DNA and RNA damage, respectively (Deng et al., 1998; Loft et al., 1995; Weimann et al., 2002). The markers can be determined by ultra-performance liquid chromatography with tandem mass spectrometric detection (UPLC–MS/MS), a technology that is highly sensitive and has been validated in international collaborations (Barregard et al., 2012; Evans et al., 2010; Henriksen et al., 2009). Increased excretion of urinary 8-oxodG have been found in experimentally induced neurodegeneration (Kikuchi et al., 2011; Yasuhara et al., 2007), as well as in clinical studies of patients suffering from neurodegenerative disorders, including dementia (Lee et al., 2007; Sato et al., 2005).

We determined the urinary excretion of 8-oxodG/8-oxoGuo by UPLC–MS/MS in severely depressed unipolar and bipolar patients (S-DEP) scheduled for electroconvulsive therapy (ECT). Samples were obtained before and 1 week after the series of ECT. We further compared baseline values with two control groups: Healthy controls (HC) and a sample of moderately depressed, non-medicated patients (M-DEP), allowing for a combined prospective and cross-sectional analysis of the relation between oxidatively generated DNA/RNA damage and depressive states. We hypothesized that the depressive state is associated with a transient increase in systemic oxidatively generated DNA and RNA damage. Specifically, we expected an increased marker excretion with increased severity of depression in the cross-sectional comparison of the groups, and a normalization of marker excretion after ECT in the S-DEP patients compared to HC.

2. Methods

2.1. Participants

Patients were recruited from on-going, mutually independent studies at Psychiatric Centre Copenhagen, Denmark, of the neurobiological and neurocognitive effects of ECT (S-DEP) and of the effect of exercise on depression (M-DEP). S-DEP patients were all in-patients at the Psychiatric Centre Copenhagen, while M-DEP patients were referred from general practices.

2.1.1. Patients with severe depression (S-DEP)

Inclusion criteria for the S-DEP patients were: Age 18–70, being able to comprehend the informed consent statement, fulfilling ICD-10 criteria for either a unipolar or bipolar depressive episode, and being eligible for ECT (as assessed by the patients' usual physician). The decision to treat the patient with ECT was not influenced by the researchers. Exclusion criteria were: significant somatic disease (e.g. heart disease, neurodegenerative disorders, cancer), alcohol or drug abuse, and coercion of any kind.

2.1.2. Patients with moderate depression (M-DEP)

Inclusion criteria for the M-DEP patients were: Age 18–60, being able to comprehend the informed consent statement, fulfilling DSM-IV criteria for major depression and having a Hamilton Depression Rating Scale 17-item (HAM-D_{17}) score >12 (see below for details). Exclusion criteria were: current drug abuse, any antidepressant medication within the last two months, current psychotherapeutic treatment, contraindications to physical exercise, more than 1 h of recreational exercise per week, suicidal behavior (HAM-$D_{17,}$ item $3>2$), pregnancy, or current/previous psychotic or manic symptoms (Krogh et al., 2012).

2.1.3. Healthy controls (HC)

HC were recruited by advertisements in the local media and from the local blood donation corps at Rigshospitalet. Exclusion criteria for the HC were current or previous psychiatric diseases and current drug abuse.

The study protocols complied with the Declaration of Helsinki, and was approved by the Regional Committee on Research Ethics (H-C-2009-005, H-3-2009-074, H-D-2008-064, H-A-2008-046). All participants gave a written informed consent before inclusion.

2.2. Diagnosis, ratings and biological samples

The diagnosis of depression was made by either an experienced psychiatrist (S-DEP), or by a structured diagnostic interview performed by a research assistant (Danish version of the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)) (M-DEP). HC were screened for the absence of psychiatric morbidity by the MINI. In all participants, depressive symptomatology was measured with the HAM-D_{17} (Hamilton, 1960). Medical doctors or research assistants at the Psychiatric Centre Copenhagen performed the HAM-D_{17} ratings. Regular joint HAM-D_{17} ratings were performed at the centre, but formal assessments of agreement was not done.

Participants were rated for subjective psychological stress by the Perceived Stress Scale questionnaire (PSS) (Cohen and Williamson, 1988). Higher PSS scores has previously been associated with oxidative stress and accelerated telomere shortening (Epel et al., 2004). In 10 simple questions, the PSS covers feelings of stress, irritability, worry etc., which are transformed into a score from zero (no stress) to 40 points (maximum stress). The timeframe was set to 2 weeks.

All patients had blood drawn for general biochemical screening, and a urine sample was obtained for the assessment of the 8-oxodG/8-oxoGuo markers. Blood and urine samples were collected in the morning in the fasting state. In previous studies, the excretion of 8-oxodG and 8-oxoGuo showed no diurnal variation (Andreoli et al., 2010). In HC and M-DEP patients, the psychometric ratings, blood and urine sampling were performed at a clinical assessment at the hospital. In S-DEP patients, ratings and samples were obtained before ECT treatments began (max. 2 days prior to the first treatment), and all ratings and samples were repeated approximately 1 week (7.8 ± 3.4 days, range 2–19 days) after the completion of ECT treatments. 28 out of 29 S-DEP patients completed the assessment 1 week post-ECT. One patient could not be reached for post-ECT assessment.

2.3. Electroconvulsive treatment

S-DEP patients received ECT following the standard protocol of the department, which comprised three treatments per week. Patients were anaesthetized by thiopental, and suxamethonium was administered for muscle relaxation. Bitemporal electrode placement was the standard. One patient received only unilateral treatments, and four patients shifted to unilateral treatments during the course of ECT due to cognitive side effects.

2.4. Urinary 8-oxodG and 8-oxoGuo

The urinary sample was obtained with a standard sampling kit without any additives. Samples were kept on ice and transferred within hours to storage at −20 °C until analysis. All samples were run at the same analytical session. At analysis, all samples were paired with samples from another group, and the pre- and post-ECT samples of the same patient where paired with each other. The urinary content of the oxidatively modified guanine nucleosides was quantified using a modification of the previously described ultra-performance liquid chromatography/tandem mass spectrometry method (Henriksen et al., 2009). Briefly, the chromatographic separation was performed on an Acquity UPLC system (Waters Corp., Milford, USA) using an Acquity UPLC BEH Shield RP18 column (1.7 μm, 2.1 × 100 mm) and a VanGuard precolumn (1.7 μm, 2.1 × 5 mm) both from Waters. The column temperature was 4 °C. The mass spectrometry detection was performed on a Xevo TQ-S triple quadrupole mass spectrometer from Waters, using electrospray ionization in the positive mode for 8-oxodG and negative ionization mode for 8-oxoGuo. The ESI(−) MS/MS transitions for 8-oxoGuo was m/z 298→208 and m/z 298→165; the corresponding transition for $^{15}N_5$-8-oxoGuo was m/z 303→213. The average *within-day* and *between-day* variation (RSD, %) estimated from the method validation was 2.3% and 9.0% for 8-oxoGuo, respectively, and 3.8% and 7.4% for 8-oxodG. The urinary creatinine concentration was determined by Jaffe's reaction. The average overall analytical variation was < 5%. The 8-oxodG/8-oxoGuo excretion is defined as the urinary concentration of the nucleoside normalised to urinary creatinine concentration. The values obtained were in agreement with the reference ranges reported by others (Andreoli et al., 2011).

2.5. Statistics

Data are presented as mean (± standard deviation) or median (interquartile range), if not otherwise stated. For analysis of the baseline group characteristics, normal distributed data were analyzed with one-way analysis of variance (ANOVA) or paired samples *t*-tests as relevant. Non-normally distributed data were analyzed with Mann–Whitney test, Kruskal–Wallis test or Wilcoxon signed rank test as relevant. For categorical data, *chi-squared* tests were used.

Due to a non-normal distribution of the 8-oxodG and 8-oxoGuo data sets, the data were transformed by the natural logarithm (Ln), which resulted in a normal distribution of the data. Two extreme values, which prevented normal transformation of the data, were excluded from the analyses. Subsequently, group comparisons of 8-oxodG/8-oxoGuo were performed by one-way ANOVA and multiple linear regression, where known confounders of oxidative stress (age, gender, body mass index and smoking status [smoker vs. non-smoker]) were included as covariates. The pre- vs. post-ECT values were analyzed by paired samples *t*-test. The associations between changes in 8-oxodG/8-oxoGuo and psychometric/ECT data were analyzed by Spearman correlations using non-transformed values. Pre- vs. post-ECT changes in medication were analyzed by Wilcoxon Signed Rank test. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 20.0 (IBM Corporation, NY, USA). Statistical significance was a priori defined as $P < 0.05$. All statistical tests were two sided.

3. Results

3.1. Basic demographical, biochemical and treatment data

Baseline data of the three participant groups are presented in Table 1, and diagnostic and treatment data of the S-DEP group are presented in Table 2. The three groups were not significantly different from each other with respect to age, gender, body mass index, blood pressure and plasma creatinine levels. There were more cigarette smokers in the M-DEP and S-DEP groups compared to HC (*chi-square* = 17.3, $P < 0.001$). The HAM-D_{17} score at baseline in the three groups were: HC = 1.0 (± 1.4) points, M-DEP = 18.6(± 2.4), S-DEP = 26.9(± 4.5) (one-way ANOVA $F(2,72) = 422.1$, $P < 0.001$).

Among the controls, one participant suffered from allergic rhinitis, and one participant had asthma. Among the M-DEP, three patients had asthma, one patient had insulin-treated diabetes, one patient had dyspepsia, and one patient had hypothyroidism. Among the S-DEP, three patients had hypertension, two patients had asthma/obstructive lung disease, one patient had hypothyroidism, and one patient was HIV-positive. In all participants, somatic conditions were pharmacologically well-controlled and in stable phase. None of the participants suffered from alcohol or drug abuse.

The average number of ECT sessions given in the S-DEP group was 12.5 (range 2–26). As expected, there was a significant reduction after ECT in both HAM-D_{17} (−15.9 [95% CI = −12.7–−19.0] points, paired samples *t*-test $t = 10.44$, $dF = 23$, $P < 0.001$) and PSS score (−10.3 [95% CI = −6.5–−14.0] points, $t = 5.6$, $dF = 25$, $P < 0.001$) (Table 1). Further, pre- vs. post-ECT differences in HAM-D_{17} and PSS scores were positively correlated (Spearman's *rho* = 0.60, $P = 0.002$). 18/28 patients responded to treatment (defined as post-ECT HAM-D_{17} < 50% of the pre-treatment value), and 9/28 achieved remission (HAM-$D_{17} \leq 7$).

There was no pre- vs. post-ECT change in medication, neither with respect to the defined daily dose (as defined by the World Health Organization) of antidepressants (Wilcoxon Signed Rank Test, $Z = -0.03$, $P = 0.98$), antipsychotics ($Z = -0.28$, $P = 0.78$), or total DDD of all medications ($Z = -0.22$, $P = 0.82$). None of the participants reported use of antioxidants.

3.2. Oxidatively generated nucleic acid damage in depressed patients and controls

The excretion of 8-oxoGuo differed between the groups (one-way ANOVA $F(2,78) = 4.6$, $P = 0.013$), with a significant trend

Table 1
Basic demographic and clinical data of the participant groups.

	HC ($N=28$)	M-DEP ($N=26$)	S-DEP ($N=29$)	P-value
Age (years)	38.9 ($\pm$13.7)	41.7 ($\pm$8.5)	46.2 ($\pm$17.0)	0.14
Gender (M/F)	11/17	11/15	14/15	0.86
Smoker (n)	3	11	13	0.001
If smoker, $<15/>15$ cigarettes per day (n)	2/1	4/7	5/8	0.62
Body mass index	26.8 ($\pm$7.6)	27.5 ($\pm$5.2)	24.3 ($\pm$4.7)	0.12
Systolic blood pressure (mmHg)	119 ($\pm$14)	117 ($\pm$ 15)	124 ($\pm$14)	0.19
Diastolic blood pressure (mmHg)	77 ($\pm$10)	80 ($\pm$10)	77 ($\pm$8)	0.62
Plasma creatinine (μmol/L)	67.2 ($\pm$10.5)	68.0 ($\pm$14.3)	72.1 ($\pm$12.6)	0.33
Previous depressive episode(s)	NA	18/26	25/29	0.10
HAM-D_{17} score pre-treatment	1.0 ($\pm$1.4)	18.6 ($\pm$2.4)	26.9 ($\pm$4.5)	<0.001[a]
HAM-D_{17} score post-treatment	NA	NA	11.7 ($\pm$6.8)	<0.001[b]
PSS score pre-treatment	7.0 ($\pm$4.8)	25.5 ($\pm$3.3)	29.1 ($\pm$3.7)	<0.001[a]
PSS score post-treatment	NA	NA	19.2 ($\pm$9.6)	<0.001[b]

Abbreviations: HC: healthy controls, M-DEP: moderately depressed patients, S-DEP: severely depressed patients treated with ECT, HAM-D_{17}: Hamilton rating scale for depression—17 item, PSS: perceived stress scale questionnaire. Data are presented as absolute numbers or means ($\pm$SD). Groups were compared with *chi-square* test, one-way ANOVA with post-hoc Tukey test, Kruskal–Wallis test or paired samples t-test, as appropriate.

[a] All groups are different from each other ($P<0.01$).
[b] Compared to pre-treatment values.

Table 2
Diagnosis and treatment data in the severely depressed patient group.

Diagnosis, ICD-10	
Unipolar/bipolar	23/6
Medication	
Antidepressants	25/29
Tricyclics	10/25
SSRI	7/25
Other	8/25
Antipsychotics	15/29
Benzodiazepines	9/29
Antiepileptics	5/29
Lithium	2/29
ECT treatment characteristics	
Previous ECT	12/29
Number of treatments given (mean (range))	12.5 (2–26)
Electrical dose, first treatment (millicoulomb, mean $\pm$ SD)	188 $\pm$ 76
Change in electrical dose during ECT course (median (range))	+58% (−25%–+400%)

towards higher levels with increased severity of depression (HC < M-DEP < S-DEP, one-way ANOVA $F(1,78)=8.8$, P for trend $=0.004$) (Fig. 1). In post-hoc analyses, 8-oxoGuo excretion was higher in S-DEP patients compared to HC (post-hoc Tukey test, $P=0.01$), but not compared to M-DEP ($P=0.15$). M-DEP and HC was not significantly different ($P=0.6$). When adjusting for age in multiple linear regression, the difference between HC and S-DEP continued to be significant ($\beta=0.25$, $P=0.04$). After the additional adjustment for other confounders of oxidative stress (gender, body mass index and smoking status), the difference between HC and S-DEP bordered on significance ($\beta=0.26$, $P=0.05$). Smoking category (no smoking, <15 or >15 cigarettes per day, see Table 1) was not significantly associated with 8-oxodG/8-oxoGuo excretion in neither group (CON, 8-oxodG: *chi-square* $=2.7$, $P=0.2$, 8-oxoGuo: *chi-square* $=2.4$, $P=0.3$. M-DEP, 8-oxodG: *chi-square* $=3.5$, $P=0.2$, 8-oxoGuo: *chi-square* $=2.3$, $P=0.3$. S-DEP, 8-oxodG: *chi-square* $=0.9$, $P=0.6$, 8-oxoGuo: *chi-square* $=2.9$, $P=0.2$).

Because bipolar disorder has also been associated with increased oxidative stress (Berk et al., 2011), we made separate analyses of the bipolar vs. the unipolar patients in the S-DEP group. When omitting the six patients with bipolar disorder from the S-DEP group, the overall difference in 8-oxoGuo excretion

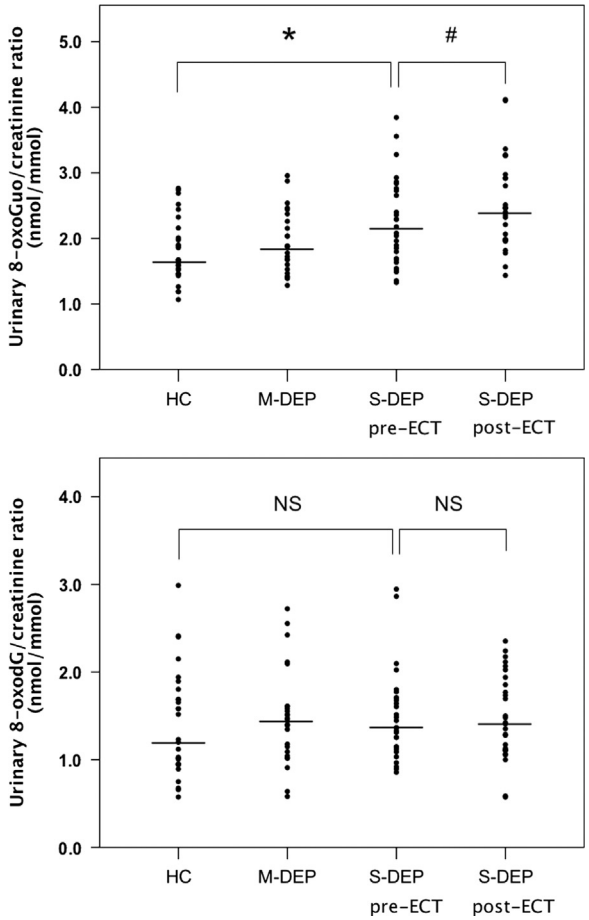

Fig. 1. Urinary excretion of 8-oxoGuo (upper panel) and 8-oxodG (lower panel) in healthy controls (HC), non-medicated moderately depressed patients (M-DEP), and severely depressed patients before (S-DEP pre-ECT) and 1 week after electroconvulsive therapy (S-DEP post-ECT). The horizontal lines indicate the median of the data. $^{*}P$ for trend $=0.004$, one-way ANOVA (Ln-transformed values). Post-hoc Tukey test of HC vs. S-DEP: $P=0.01$, M-DEP vs. S-DEP: $P=0.15$, HC vs. M-DEP: $P=0.6$. $^{\#}P=0.006$, paired samples t-test (Ln-transformed values). There are no significant differences in 8-oxodG excretion between the groups or pre- vs. post-ECT.

between the three groups was no longer significant ($F(2,72)=2.20$, $P=0.1$), while the increased levels with increased severity of depression continued to be significant ($F(1,78)=4.40$, P for trend $=0.04$). In a separate analysis of the S-DEP patients, 8-oxoGuo excretion was higher in bipolar vs. unipolar patients (Mann–Whitney U (MWU)$=31.0$, $P=0.04$).

The excretion of 8-oxodG was not significantly different between the groups, either before (one-way ANOVA $F(2,78)=0.44$, $P=0.65$) nor after adjustment for the abovementioned confounders in multiple linear regression analysis ($\beta=0.003$, $P=0.98$) (Fig. 1). Omitting the bipolar S-DEP patients from analysis did not alter the result ($F(2,73)=0.45$, $P=0.64$), and 8-oxodG excretion was not different in bipolar vs. unipolar patients ($MWU=67.0$, $P=0.91$).

When analyzing the groups separately, there were no significant associations between 8-oxodG/8-oxoGuo excretion and HAM-D_{17} or PSS score. However, when analyzing the entire population as a whole, there was a significant positive association between 8-oxoGuo excretion and PSS score ($rho=0.30$, $P=0.01$), and a borderline significant correlation with HAM-D_{17} score ($rho=0.22$, $P=0.06$).

In the S-DEP patients, there were no significant associations between neither marker and measures of medication load: Number of antidepressants used (8-oxodG: one-way ANOVA $F(3,25)=1.50$, $P=0.2$, 8-oxoGuo: $F(3,25)=0.30$, $P=0.8$), DDD of ADs (8-oxodG: $rho=$-0.19, $P=0.3$, 8-oxoGuo: $rho=-0.11$, $P=0.6$), and total DDD of all medications (8-oxodG: $rho=$-0.18, $P=0.3$, 8-oxoGuo: $rho=-0.20$, $P=0.4$). There was no difference in 8-oxodG/8-oxoGuo excretion in patients taking selective serotonin reuptake inhibitors ($N=18$) vs. patients taking tricyclic antidepressants ($N=11$) (8-oxodG: $MWU=90.0$, $P=0.7$, 8-oxoGuo: $MWU=92.0$, $P=0.8$). Further, there was no difference in 8-oxodG/8-oxoGuo excretion in patients taking benzodiazepines ($N=10$) vs. those who did not ($N=19$) (8-oxodG: $MWU=57.0$, $P=0.09$, 8-oxoGuo: $MWU=62.0$, $P=0.1$). In patients taking antipsychotics ($N=15$) vs. those who did not ($N=14$), we found no difference in 8-oxoGuo excretion ($MWU=100.0$, $P=0.8$), and a significantly higher 8-oxodG excretion in antipsychotic users ($MWU=48.0$, $P=0.01$), which could both be attributed to the medication and/or the presence of psychosis.

3.3. Oxidatively generated nucleic acid damage before and after electroconvulsive therapy

The urinary excretion of 8-oxoGuo was further increased after ECT (pre-ECT: 2.18 (1.69–2.80) nmol/mmol creatinine, post-ECT: 2.42 (1.97–2.96) nmol/mmol creatinine, paired samples t-test on Ln-transformed values, $t=3.0$, $dF=27$, $P=0.006$). In contrast, there was no change in 8-oxodG excretion (pre-ECT: 1.36 (1.13–1.71) nmol/mmol creatinine, post-ECT: 1.41 (1.11–1.92) nmol/mmol creatinine, $t=-0.2$, $dF=27$, $P=0.8$) (Fig. 1). Pre- to post-ECT differences in the 8-oxodG and 8-oxoGuo excretion were not correlated to measures of treatment response, including change in HAM-D_{17} (8-oxodG: Spearman's $rho=-0.09$, $P=0.7$, 8-oxoGuo: $rho=-0.1$, $P=0.6$) or PSS (8-oxodG: $rho=-0.06$, $P=0.8$, 8-oxoGuo: $rho=-0.03$, $P=0.9$). This was also the case when patients were dichotomized by HAM-D_{17} reduction into responders and non-responders, or remitters and non-remitters (results not presented).

There were no significant correlations between changes in marker excretion and ECT treatment characteristics, such as the number of treatments given (8-oxodG: $rho=-0.33$, $P=0.1$, 8-oxoGuo: $rho=-0.23$, $P=0.2$), energy applied at last ECT (8-oxodG: $rho=$-0.25, $P=0.9$, 8-oxoGuo: $rho=0.19$, $P=0.9$), energy increase during the course of ECT (8-oxodG: $rho=-0.15$, $P=0.5$, 8-oxoGuo: $rho=-0.07$, $P=0.8$), or number of days from the last ECT to urinary sampling (8-oxodG: $rho=-0.01$, $P=0.98$, 8-oxoGuo: $rho=-0.01$, $P=0.96$).

4. Discussion

To our knowledge, this is the first study to demonstrate an increased systemic RNA damage from oxidation in severe depression, and the first clinical study to measure markers of oxidative stress after ECT for depression. 8-oxoGuo excretion was higher with increasing severity of depression (HC < M-DEP < S-DEP), and in post-hoc comparisons, we found a significantly elevated 8-oxoGuo excretion in S-DEP patients compared to HC. After the adjustment for key confounders of oxidative stress (age, gender, body mass index and smoking), this difference bordered on significance ($P=0.05$), suggesting that disparities in these variables among the groups may explain some, albeit not all, of the difference in systemic oxidative stress on RNA. Contrary to our hypothesis, 8-oxoGuo excretion in the S-DEP patients was further increased after ECT.

Although the M-DEP patients appeared to have an intermediate level of urinary 8-oxoGuo excretion, the differences between the M-DEP and the HC or S-DEP groups did not reach statistical significance in post-hoc comparisons. A possible interpretation of this finding is that a depressive disorder per se is not sufficient to substantially increase systemic oxidative stress on RNA, but require the serious physical strains (e.g. HPA-axis disturbances and prolonged physical inactivity) associated with the severely depressed state. Also, the difference between the levels of 8-oxoGuo excretion in the severely and the moderately depressed patients might be due to the nature of the depression in the two patient categories, rather than just the severity of depression as measured by HAM-D_{17}. The patients given ECT thus generally had symptoms of an endogenous/melancholic type of depression, while this was not predominant in the M-DEP patient group, which was referred from general practitioners. It can therefore not be ruled out that fundamental differences in pathophysiology between the patient groups may play a role for the differential findings.

The oxidation of mRNA causes translation errors and reduced protein synthesis (Shan et al., 2007; Tanaka et al., 2007), while the oxidation of non-coding RNA species may affect the regulation of protein translation, gene expression, and neuronal synaptic plasticity (Satterlee et al., 2007). In humans, oxidatively generated damage to RNA has been suggested as a pathogenic mechanism in age-related disorders such as Alzheimer's disease (Nunomura et al., 2009) and type 2 diabetes (Broedbaek et al., 2011). Hence, oxidatively generated RNA damage could be an additional mechanism underlying the neurobiological abnormalities and somatic comorbidity associated with severe depression.

In contrast to the finding of others (Forlenza and Miller, 2006; Irie et al., 2005; Maes et al., 2009), we were unable to detect any differences between the groups in 8-oxodG, i.e. systemic DNA damage from oxidation, in depressed individuals. This discrepancy could be due to methodological differences, in that 8-oxodG in the abovementioned studies was measured in serum/urine by ELISA or in nuclear DNA from leukocytes by liquid chromatography with electrochemical detection, whereas we measured 8-oxodG in urine by UPLC/MS-MS. Although the accuracy of ELISA methods in determining 8-oxodG has recently been questioned (Barregard et al., 2012; Garratt et al., 2010), the study by Forlenza and Miller included a considerably larger number of patients ($N=169$) and controls ($N=85$), raising the possibility that our null finding represents a type II error. However, in line with our finding, a recent large study of Japanese office workers did not show any significant associations between oxidatively generated DNA damage, as measured by spot urine 8-oxodG determined by

liquid chromatography, and depressive symptoms (Yi et al., 2012).

We determined the 8-oxodG and 8-oxoGuo markers after ECT in the S-DEP group. One previous study found a decrease in malondialdehyde (MDA, a measure of lipid peroxidation) in schizophrenia patients after a series of 9 ECT treatments (Kartalci et al., 2011). ECT has repeatedly been found to be superior to pharmacotherapy in the treatment of severe depression (Group, 2003), and results in a rapid improvement of depressive symptoms in the majority of patients (Husain et al., 2004). Furthermore, ECT may exert its action through a normalization of HPA-axis activity (Bolwig, 2011), which has been associated to oxidatively generated nucleic acid damage in both animal and human studies (Caro et al., 2007; Joergensen et al., 2011). The response and remission rates were of the same size as in previous studies (Medda et al., 2009). However, in spite of a substantial reduction in depressive symptomatology and perceived stress after ECT, the 8-oxodG marker showed no change, and 8-oxoGuo exhibited a small but significant increase. This finding suggests that RNA oxidation damage is not causally linked to the depressed state per se, i.e. that increased systemic RNA damage from oxidation in severely depressed individuals is a trait rather than a state phenomenon. Furthermore, we recently found urinary 8-oxoGuo (as well as 8-oxodG) excretion to be increased in schizophrenia patients compared to healthy controls (Jorgensen et al., 2013), suggesting that within the context of mental disorders, elevated levels of systemic RNA oxidation are not specific to depression.

The current finding raises the possibility that ECT in itself causes increased oxidative stress to RNA. Animal models of ECT provide some evidence for an increased oxidatively generated damage to lipids and proteins in the frontal cortex after multiple electroconvulsive stimulations (ECS), with conflicting results for other brain regions such as the hippocampus and striatum (Barichello et al., 2004; Feier et al., 2006; Jornada et al., 2007; Zupan et al., 2008). However, nucleic acid damage from oxidation remains to be explored in these ECS models.

ECT elicits a substantially increased binding of the transcription factor cyclic adenosine monophosphate response element binding protein (CREB) to its promotor regions (Tanis et al., 2008), thus possibly raising the absolute number of RNA transcripts available for oxidation in the brain. ECT-induced increases in oxidative stress and RNA synthesis could - alone or in combination - increase the output of oxidized RNA nucleosides from the brain, which is subsequently recovered in urine. However, within the context of our study, we found no significant correlations between the pre- to post-ECT change in marker excretion and ECT treatment characteristics, including number of treatments, applied electrical dose at final treatment, and electrical dose increase during the course of ECT (a measure of increased seizure threshold). There was also no association between the change in marker excretion and the time passed from the last ECT to post-ECT sampling.

The theoretical possibility that increased RNA oxidation is a mechanism by which ECT works should also be considered. While the fate of oxidized RNA in the cell is far from clarified, evidence suggests that oxidatively modified RNA is not repaired by specific enzymes, as is the case for DNA, but may undergo targeted enzymatic degradation (Li et al., 2006). Depression has been associated with a range of gene expression changes in the brain, some of which appear to be causally involved in the pathogenesis of the disease (Sun et al., 2012). It is possible that an increased oxidation and subsequent degradation of such a "depressogenic transcriptome" could have antidepressant effects. However, this is highly speculative and not directly supported by our findings. The abovementioned issues could be further addressed by measuring the 8-oxodG/8-oxoGuo markers in other treatment modalities for depression, such as pharmacotherapy and psychotherapy, and in animal models of ECT.

We found a discrepancy in the excretion of the RNA and the DNA oxidation marker, both between the groups and in relation to ECT in the S-DEP patients. RNA is single-stranded, not protected by specific proteins, and situated in the proximity of the main cellular source of reactive oxygen species, the mitochondria, and therefore may be particularly vulnerable to increased oxidative stress levels (Kong and Lin, 2010). Interestingly, a recent study comparing post-mortem hippocampal levels of intracellular DNA and RNA oxidation levels in schizophrenia, bipolar disorder, depression, and healthy controls, found that in all psychiatric disorders, RNA - but not DNA - oxidation were increased (Che et al., 2010).

There are several limitations to the study. First, the number of participants in each group was relatively low, limiting our ability to adjust for potential confounders, and raising the possibility that the null findings (such as the absence of differences between the groups in 8-oxodG excretion, and between the HC and M-DEP groups in 8-oxoGuo excretion) could be due to type II errors. Therefore, these results should be regarded as preliminary and need to be replicated in a larger sample. Second, in contrast to the M-DEP group, the S-DEP patients consisted of both unipolar ($N=23$) and bipolar ($N=6$) patients, and omitting the bipolar patients from analysis significantly influenced the results, reflecting a significantly higher 8-oxoGuo excretion in the bipolar patients. Given the low number of participants in each disease category, these subanalyses should be interpreted with caution, and we are not able to further address the potential discrepancy between oxidative stress on RNA in unipolar vs. bipolar disorder within the context of this study. Third, S-DEP patient were medicated, and although we found no associations between the 8-oxodG/8-oxoGuo excretion and measures of overall medication load, an effect of medication cannot be ruled out. However, given that ECT is a treatment option reserved for the severely ill, it is not feasible to recruit a sample of non-medicated ECT patients. Fourth, the study was based on samples from otherwise independent studies, and therefore in- and exclusion criteria, as well as the collection of baseline demographical and biochemical data, were not completely coordinated. Finally, it is possible that there is a temporal mismatch between the symptom relief observed after ECT and a reduction/inhibition of (neuro)degenerative processes suggested to occur during depressive episodes (Moylan et al., 2012). Hence, the sampling of urine immediately before and 1 week after the completion of ECT does not span the entire depressive episode, and it could be suspected that a longer follow-up period would have revealed a normalization of the oxidation markers.

In conclusion, we found that systemic oxidatively generated RNA damage, as measured by the urinary excretion of 8-oxoGuo, was increased in severely depressed patients eligible for ECT in comparison to healthy controls, and showed a trend towards an increase in comparison to moderately depressed, non-medicated patients. The increase was not normalized 1 week after clinically successful ECT, but on the contrary exhibited a small but significant further increase. Levels of systemic DNA damage, as measured by the excretion of 8-oxodG, were not different among the groups or before vs. after ECT. The study highlights a possible role for systemic RNA oxidation as an instigator of medical morbidity in affective disorders, but also brings into question the extent to which oxidative stress is related to the pathogenesis of depression.

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Conflict of interest

None of the authors report any conflicts of interest.

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