Increased Serum Levels of 8-Hydroxy-2′-Deoxyguanosine in Clinical Depression

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Objective: We sought to understand the pathophysiological effects of depression by examining group differences in serum levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative damage. **Methods:** Our sample consisted of 169 participants. Eight-four of these participants met diagnostic criteria for clinical depression. The 85 participants in our comparison group were matched on age, gender, and ethnicity to the depressed group. 8-OHdG was measured by enzyme-linked immunosorbent assay. **Results:** After adjusting for age, gender, race/ethnicity, years of education, daily smoking, average number of alcoholic drinks per week, average amount of physical activity per week, and body mass index, participants in the depressed group had significantly higher levels of oxidative DNA damage compared with participants in the control group. Pairwise comparisons showed that participants with major depression had significantly higher levels of 8-OHdG than control subjects and marginally higher levels of 8-OHdG compared with those with minor depression. Furthermore, participants with recurrent episodes of depression had more oxidative damage than participants with single episodes, who in turn had more damage than healthy control subjects. Finally, participants with recurrent episodes of major depression had more DNA damage than other depressed participants, who in turn had more damage than healthy control subjects. **Conclusions:** Our findings suggest that increased oxidative damage may represent a common pathophysiological mechanism, whereby depressed individuals become vulnerable to comorbid medical illness. **Key words:** depression, DNA damage, oxidative damage, comorbidity.

8-OHdG = 8-hydroxy-2′-deoxyguanosine; **DSM-IV** = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; **DISH** = Depression Interview and Structured Hamilton Interview; **ELISA** = enzyme-linked immunosorbent assay; **HPLC-EC** = high-performance liquid chromatography with electrochemical detection; **GC-MS** = gas chromatography–mass spectrometry; **BMI** = body mass index.

INTRODUCTION

Clinical depression is a considerable public health problem (1). Approximately 32 to 35 million adults in the United States have experienced depression at some point in their life and approximately 13 million adults have experienced depression within the past year (2). Clinical depression is also a considerable medical problem as those with major depressive disorder (MDD) are at increased risk for serious medical illness, including cardiovascular disease (3–5), diabetes (6–8), cancer (9–11), and stroke (12). This risk is often independent of traditional risk factors, suggesting that depression may function as a causal factor in the pathogenesis of multiple diseases. To understand the pathophysiological effects of depression, we examined one of the biologic mechanisms common to multiple diseases: oxidative damage to DNA.

Oxidative damage results from biochemical interactions between reactive oxygen species (ROS) and target biomolecules. ROS can damage nucleic acids, lipids, and proteins; this damage figures prominently in the etiology and progression of numerous cancers (13–15) as well as coronary and carotid atherosclerosis (16–19). Although many damaged DNA lesions have been identified (20–23), we have chosen 8-hydroxy-2′-deoxyguanosine (8-OHdG) as our biomarker of oxidative damage. The importance of this lesion stems from the fact that it is both abundant in DNA and it is mutagenic (24–28). Current evidence suggests that 8-OHdG lesions present in DNA during cellular replication results in somatic mutation, the driving force behind carcinogenesis. Emerging evidence suggests that somatic mutation may contribute to smooth muscle proliferation in the pathogenesis of atherosclerotic plaques (29,30).

Research investigating associations between psychologic factors and DNA damage is a relatively new area of study, and there are no published studies examining oxidative damage levels among clinically depressed individuals. The primary purpose of this article was to compare mean levels of oxidative DNA damage between clinically depressed patients and a matched comparison group. We were interested in answering several related questions: 1) Do patients with depression have higher levels of oxidative DNA damage, as reflected by 8-OHdG, compared with healthy control subjects? 2) Is there a dose–response relationship between the severity of depressive symptoms and the extent of DNA damage? 3) Do patients with longer-standing problems with depression, i.e., those with recurrent episodes, have greater oxidative damage than patients with a single episode of depression? To our knowledge, this study represents the first attempt to quantify oxidative DNA damage among patients with clinical depression.

METHODS

Participants

All volunteer participants, both depressed and control subjects, were recruited from January 2001 to May 2003 by advertisements placed in mass transit stations and newspapers as well as posted flyers in the St. Louis metropolitan area. Eighty-four of the participants met diagnostic criteria for clinical depression and constituted our depressed group. None of these participants were taking antidepressant medication for their current depressive episode. Our comparison group consisted of 85 participants and was matched case-by-case to the depressed group on age, gender, and ethnicity. All participants were medically healthy at study entry: They had no history of chronic medical illness, no indications of acute infection, normal complete

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blood count, and no prescribed medication regimen other than oral contraceptives in the last 6 months. Additionally, participants in the comparison group had no history of psychiatric illness. Each participant had a complete blood count (CBC) with differential to screen for acute infection (Beckman Coulter, Fullerton, CA). Serum albumin was determined by spectrophotometry (Kyowa Medes, Shizuoka, Japan) and used as a measure of gross nutritional status to exclude subjects with protein-store deficiencies (there were none). Using such a medically healthy sample allowed us to rule out residual confounding by prevalent disease as an explanation for our effects.

Assessment of Depression

All participants in the depressed group met diagnostic criteria for a current major depressive disorder (MDD) or minor depressive disorder as defined in the DSM-IV (31). Diagnoses were made by trained interviewers using the Depression Interview and Structured Hamilton Interview (DISH), a semi-structured interview that provides both DSM-IV major and minor depressive disorder diagnoses and a severity score based on the Structured Interview Guide for the Hamilton Depression scale (32). To meet criteria for current MDD, participants must report at least one of the following: depressed mood or loss of interest or pleasure (31). Furthermore, a participant had to have at least five out of a possible nine symptoms in the past 2 weeks; these symptoms must cause clinically significant distress or impairment. Symptoms cannot result from substance abuse, medication, or a general medial condition and cannot be accounted for by bereavement. Minor depression is similar to MDD in duration but is less severe or impairing (two rather than five symptoms are required). To meet criteria for recurrent MDD, participants must have experienced at least two separate episodes of MDD with at least two consecutive months in between (31).

Participants were excluded if they had comorbid psychotic disorders, eating disorders, substance abuse disorders, or anxiety disorders other than generalized anxiety disorder. Determination of exclusionary comorbid psychiatric disease was made using modified versions of the Diagnostic Interview Schedule and the Primary Care Evaluation of Mental Disorders (33,34).

Procedures

Sessions began with the explanation of study details and the collection of written informed consent. To determine eligibility, potential participants underwent the DISH. The interviewers received extensive training on the DISH before the study began. To assess the extent of their diagnostic agreement, both interviewers rated a series of 20 participants. Across the 11 symptom dimensions of the DISH, the interviewers showed an average kappa of 0.79, a value indicating good to excellent diagnostic agreement. Eligible participants then answered a series of questionnaires regarding their medical history and personal health behaviors. Their height and weight was measured for calculation of body mass index (BMI). Participants next provided a 35-mL blood sample by antecubital venipuncture. Blood samples were centrifuged at 1000 g for 25 minutes and then the serum was aspirated, divided into aliquots, and stored at −70°C until the end of the study. All blood draws were taken in the early morning to control for diurnal rhythms.

Measure of Oxidative Damage

We used serum levels of the oxidized base, 8-hydroxy-2′-deoxyguanosine (8-OHdG), as our biomarker of oxidative damage. 8-OHdG was measured with the highly sensitive 8-OHdG check enzyme-linked immunosorbent assay (ELISA) kit (Genox Corp., Baltimore, MD). This competitive in vitro ELISA is specifically designed for measurement of 8-OHdG in tissues expected to have low levels of this damage lesion. Antibody techniques like ELISA offer a valid and comparatively simple alternative to more technically demanding HPLC-EC or GC-MS techniques for the quantitation of oxidative DNA damage (35), and research suggests good correlation between HPLC and ELISA methods ($r = 0.96$; [36]).

Procedures followed manufacturer's instructions. First, serum samples were passed through Amicon Ultra Centrifugal Filters (Fisher Scientific) to remove any large-molecular-weight substances. Samples and standards (50 μL) were then added to microtiter plates precoated with 8-OHdG. This was followed by the addition of 8-OHdG monoclonal antibodies (50 μL). Plates were then sealed and incubated at 4°C overnight. In this primary reaction, the 8-OHdG in the serum competes with the 8-OHdG already bound to the plate for the monoclonal antibody. Higher levels of 8-OHdG in the sample will lead to lower levels of antibody binding to the plate.

To remove the antibodies bound to 8-OHdG in the serum, the plates were washed with 250 μL diluted washing buffer and a second enzyme-labeled antibody (100 μL) was added to each well. This second antibody will bind to the 8-OHdG monoclonal antibody already attached to the plate. After repeating the plate wash, 100 μL of chromogen was added to each well and incubated in the dark for 15 minutes. The development of color is proportional to the amount of antibody in the plate, which in turn is inversely related to the amount of 8-OHdG in the serum sample. Lower color means higher amounts of 8-OHdG. Results are expressed in nanograms per milliliter.

Samples from each subject were split and run in triplicate. Plates were read by a Tecan Sunrise microplate reader. The concentration of 8-OHdG in each sample was determined by generating standard curves for each lot of assay reagents from standardized samples contained in each ELISA kit. Curve fitting was done with Magellan 4.0 software using a four-point logistic model. The mean of each subject's three samples was computed and then converted into a z-score to facilitate interpretation of statistical analyses.

The intraassay coefficient of variation was an acceptable 10.8% (37). We interpret measurements of 8-OHdG in serum as reflective of overall oxidative DNA damage in the whole body, similar to the interpretation of 8-OHdG excreted in urine (38). This measurement will include not only damaged nuclear DNA removed during base excision repair, but also damaged mitochondrial DNA and damaged DNA from cytoplasmic nucleotide pools (35).

Covariates

Gender, ethnic/racial identity, and years of education were all self-reported. If subjects reported that they smoked cigarettes daily, they were classified as regular smokers. Body mass index was calculated from height and weight measurements taken at each session using a balance beam scale (Seca Corp., Columbia, MD) and were averaged across sessions. Alcohol consumption was defined as the reported average number of alcoholic drinks per week. Physical activity was measured with the Paffenbarger Physical Activity Scale and was defined as the minutes per week the subject participates in any regular activity akin to brisk walking, jogging, bicycling, and so on, long enough to work up a sweat. This scale item is the best self-reported predictor of treadmill-tested VO2-max in healthy men (39).

Statistical Analyses

We conducted statistical analyses with SPSS (version 10.1.1; SPSS, Inc., 2000). Preliminary analyses included a series of one-way analysis of variance (ANOVA) or χ^2 tests of association to determine group equivalence. Variables that were significantly different between groups were entered in subsequent analyses as covariates. Additional covariates were selected based on published reports of associations with oxidative DNA damage (21,40–51). Estimated mean differences in serum levels of 8-OHdG were determined by analysis of covariance (ANCOVA) with age, gender, ethnicity, years of education, daily smoking, average number of alcoholic drinks per week, average amount of brisk physical activity per week, and BMI entered as covariates. When depression was stratified into more than two categories, we used the polynomial linear contrast procedure to evaluate linear trends across groups. Significant trend tests were followed by pairwise comparisons.

RESULTS

Our final sample consisted of 169 people, approximately 81% of whom were women. As expected, depressed and comparison participants were similar on most demographic variables, because they were matched during the selection process on age, gender, and ethnicity. Exceptions are as follows: there was a significantly higher proportion of smokers in the depressed group than in the comparison group (χ^2 [1] = 18.89, $p < .001$). Also, depressed participants were significantly less educated (F [1, 167] = 12.93, $p < .001$) and were

marginally heavier than comparison participants (F [1, 166] = 3.64, $p < .06$). Finally, the distribution of income was different across groups: there were more depressed people in the lower income groups than nondepressed people and there were more nondepressed people in the upper income groups than depressed people (Mann-Whitney U test, $p < .05$).

Participants in the depressed group had a mean Hamilton depression score of 19.0 ± 5.1. In contrast, participants in the comparison group had a mean Hamilton score of 0.4 ± 0.9 and none were categorized as depressed by the Hamilton Depression Scale. The partial correlation between Hamilton depression scores and serum levels of 8-OHdG was small (r = 0.15, $p = .07$) controlling for age, gender, race/ethnicity, years of education, daily smoking, average number of alcoholic drinks per week, average amount of physical activity per week, and BMI (Table 1).

Group Differences in Oxidative DNA Damage

After adjusting for age, gender, race/ethnicity, years of education, daily smoking, average number of alcoholic drinks per week, average amount of physical activity per week, and BMI, participants in the depressed group had significantly higher levels of oxidative DNA damage, as reflected in serum 8-OHdG, compared with participants in the control group (F [1, 153] = 4.83, $p = .029$).[1] This disparity was fairly large; after the means had been adjusted for covariates, depressed patients' 8-OHdG levels were nearly 0.4 standard deviations higher than control subjects (Figure 1).

TABLE 1. Sample Demographics and Characteristics

	Depressed (n = 84)	Comparison (n = 85)
Age (yrs ± SD)	28.7 ± 9.0	28.9 ± 8.9
Women, *n* (%)	68 (81)	69 (81.2)
White, *n* (%)	39 (46.4)	40 (47.1)
Black, *n* (%)	38 (45.2)	38 (44.7)
Other, *n* (%)	7 (8.4)	7 (8.3)
Smokers,[a] *n* (%)	27 (32.1)	5 (5.9)
Body mass index (mean ± SD)	29.2 ± 9.7	26.8 ± 6.5
Obese, *n* (%)	33 (39.3)	21 (25.0)
Average drinks per week (mean ± SD)	3.8 ± 7.4	2.70 ± 5.1
Years of education[b] (mean ± SD)	14.2 ± 2.3	15.4 ± 1.9
Last year household income (median category)[c]	$10,000–14,999	$20,000–29,999
Brisk physical activity (minimum per week ± SD)	89.6 ± 174.5	111.5 ± 172.8
Serum 8-OHdG (mean ± SD) (untransformed)	0.72 (0.29) ng/mL	0.64 (0.23) ng/mL
Serum 8-OHdG (range) (untransformed)	0.27–1.70 ng/mL	0.20–1.26

[a] χ^2 (1) = 18.89, $p < .001$.
[b] t (167) = 3.60, $p < .001$.
[c] Mann-Whitney U test, $p = .049$.
SD = standard deviation.

Oxidative Damage and Severity of Depression

To determine if there was a dose–response relationship between severity of depression and oxidative damage, we stratified the depressed group into those with major (n = 62) or minor (n = 22) depression according to DSM-IV criteria. Participants with major depression had an unadjusted mean (standard deviation [SD]) level of 8-OHdG of 0.75 (0.32) ng/mL and participants with minor depression had an unadjusted mean (SD) level of 8-OHdG of 0.63 (0.19) ng/mL. Adjusting for age, gender, race/ethnicity, years of education, daily smoking, average number of alcoholic drinks per week, average amount of physical activity per week, and BMI, an omnibus test showed significant group differences in levels of 8-OHdG (F [2, 152] = 4.22, $p = .016$). A polynomial linear contrast was also significant (contrast estimate [standard error [SE]] = 0.362 [0.130], $p = .006$). Pairwise comparisons showed that participants with major depression had significantly higher levels of 8-OHdG than controls (F [1, 141] = 7.81, $p = .006$) and marginally higher levels of 8-OHdG compared with those with minor depression (F [1, 80] = 3.54, $p = .062$). This likely was the result of low power resulting from the small number of participants with minor depression. Participants with minor depression did not reliably differ from controls (F [1, 101] = 0.007, $p = .933$). The effects here were also fairly large; after the means had been adjusted for covariates, participants' with major depression had levels of 8-OHdG that were nearly 0.5 standard deviations higher than participants with minor depression and control subjects.

Oxidative Damage and History of Depression

We next sought to determine if those with a history of recurrent depression (n = 59) evidenced higher levels of 8-OHdG compared with those with either a single episode of depression (n = 25) or no depression (n = 85). Participants with recurrent depression had an unadjusted mean (SD) level of 8-OHdG of 0.73 (0.31) ng/mL and participants with a single episode of depression had an unadjusted mean (SD) level of 8-OHdG of 0.68 (0.25) ng/mL. After adjusting for covariates, an omnibus test showed a marginal difference between the groups (F [2, 152] = 2.54, $p = .08$). Again, polynomial linear contrast analysis yielded evidence of a significant linear trend for history of depression (contrast estimate [SE] = 0.287 [0.129], $p = .027$) such that participants with recurrent episodes had greater oxidative damage

[1] Nineteen (11%) of 169 participants were missing data on income and the missing data were not balanced across groups; income data was missing for 14 participants from the control and five participants from the depressed group. An analysis of covariance analysis, including income as an additional covariate yielded identical results to those presented in the text (F = 5.01, p = .027). Therefore, to retain as many participants as possible, we did not include income as a covariate in subsequent analyses.

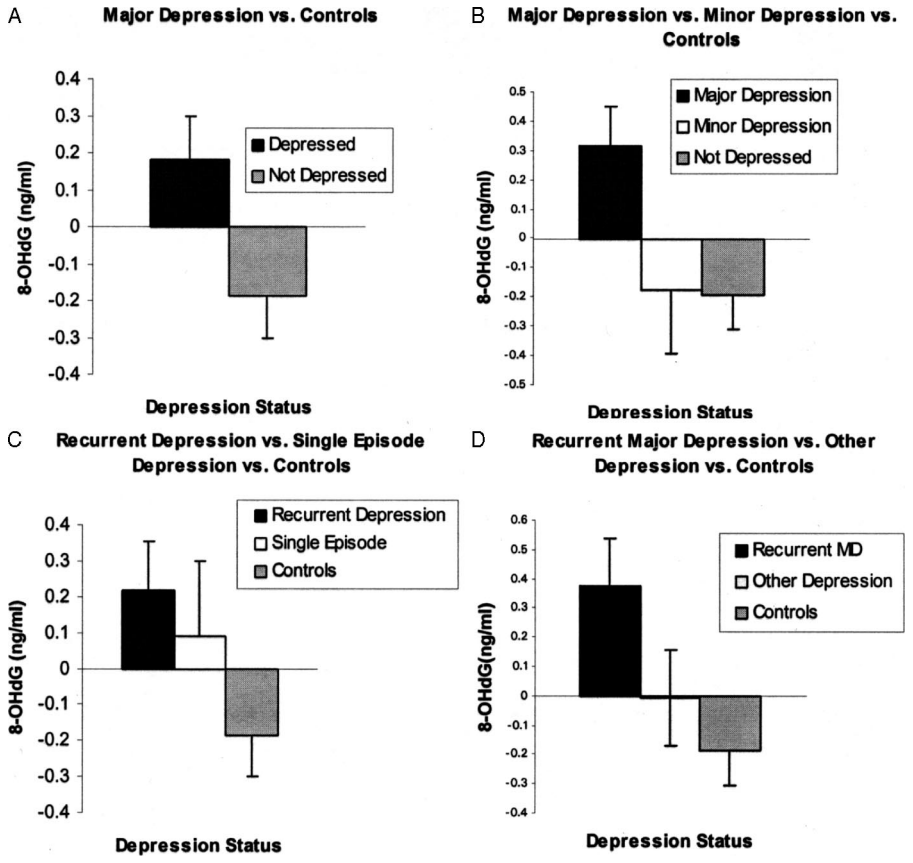

Figure 1. Estimated marginal means (standard error of mean) of serum levels of 8-OHdG by depression status controlling for age, gender, race/ethnicity, years of education, average number of alcoholic drinks per week, average amount of physical activity per week, and body mass index. (**A**) Mean level of 8-OHdG in serum of depression and nondepressed participants (F [1, 153] = 4.83, p = .029). (**B**) Mean Level of 8-OHdG in serum of participants with major depression, minor depression, and no depression. Univariate tests showed significant group differences in levels of 8-OHdG (F [2, 152] = 4.22, p = .016). A polynomial linear contrast was significant (contrast estimate [SE] = 0.362 [0.130], p = .006). (**C**) Mean level of 8-OHdG in serum of participants with recurrent depression, a single episode of depression, and no depression. Univariate tests showed was a marginal difference between the groups (F [2, 152] = 2.54, p = .08). Polynomial linear contrast analysis showed a significant linear trend for history of depression (contrast estimate [SE] = 0.287 [0.129], p = .027). (**D**) Mean Level of 8-OHdG in serum of participants with recurrent major depression, other depression, and no depression. Univariate test showed significant differences in levels of 8-OHdG between the groups (F [2, 152] = 3.83, p = .024). A polynomial linear contrast showed a significant linear trend (contrast estimate [SE] = 0.399 [0.144], p = .006).

than participants with single episodes who in turn had more damage than healthy control subjects. These effects were independent of all covariates we included. The differences here were more moderate; after means were adjusted for covariates, participants with recurrent depression had 8-OHdG levels were 0.1 SD higher than participants with a single episode of depression and participants with a single episode of depression had 8-OHdG levels that were 0.3 SD higher than those without depression. Those with recurrent depression had levels of 8-OHdG that were 0.4 SD higher than control subjects.

Finally, we examined whether oxidative damage was especially pronounced in those participants who experience more severe mood problems, which is often defined as having recurrent episodes of major depression (52). Participants with recurrent major depression (n = 41) had an unadjusted mean (SD) level of 8-OHdG of 0.77 (0.34) ng/mL and other depressed participants (n = 43) had an unadjusted mean (SD) level of 8-OHdG of 0.67 (0.23) ng/mL. An omnibus test showed significant differences in levels of 8-OHdG between the groups (F [2, 152] = 3.83, p = .024). A polynomial linear contrast showed a significant linear trend for this variable (contrast estimate [SE] = 0.399 [0.144], p = .006) such that participants with recurrent episodes of major depression had more DNA damage than other depressed participants who in turn had more damage than nondepressed control subjects. These effects were independent of all covariates we included. After means were adjusted for covariates, participants with recurrent major depression had 8-OHdG levels were 0.4 SD higher than other depressed participants who in turn had 8-OHdG levels that were 0.2 SD higher than those without control subjects.

DISCUSSION

This study represents the first attempt to assess levels of the oxidative damage lesion 8-OHdG in the serum of clinically depressed but otherwise healthy adults. As our results demonstrate, compared with matched control subjects, those with clinical depression show 0.4 SD higher serum levels of 8-OHdG. Furthermore, there appears to be a relationship be-

tween severity of depression and level of 8-OHdG: those with major depression have levels of 8-OHdG 0.5 SD higher than those with either minor depression or no depression; those with recurrent depression have levels of 8-OHdG 0.4 SD higher than those with no depression; and those with severe depression (recurrent major episodes) have levels of 8-OHdG 0.4 SD higher than those with a single episode of major depression, recurrent or a single episode of minor depression, and 0.5 SD higher than those with no history of depression.

Our results are consistent with the work of Irie and colleagues who conducted a number of studies examining correlations among negative mood, depressive symptoms, and oxidative DNA damage in healthy workers without clinical depression. Among women, positive correlations were found between levels of 8-OHdG and 1) negative mood as measured by Profile of Mood States (53); 2) perceived workload, perceived psychological distress, and the perceived impossibility of alleviating distress (54); and 3) severe depression as measured by the General Health Questionnaire (55). Although these studies were conducted with healthy individuals and not clinically depressed patients, these preliminary but provocative findings suggest that oxidative damage may exist on a continuum and underscores the need to further examine levels of oxidative DNA damage in clinically depressed people.

Patients with MDD are at increased risk for a variety of chronic diseases (56). Our findings suggest that the clinical depression associated with increased oxidative damage may represent a common pathophysiological mechanism whereby these patients become vulnerable to multiple comorbid medical illness. We believe that chronic and severe depression contributes to persistent oxidative stress that in time overwhelms protective molecular and cellular mechanisms and simultaneously increases oxidative damage to important macromolecules, including lipids, proteins, and DNA. To the extent that this it true, differences in the accumulating damage to lipids, proteins, and nucleic acids, in conjunction with other genetic and behavioral susceptibility factors, would determine which disease emerges over time. This model remains to be fully tested.

It is not immediately clear from the present research how depressed people might come to have higher levels oxidative damage, although there are several possibilities. First, clinically depressed people may alter their behaviors in such a way as to increase oxidative damage. For example, among smokers, depression may lead to increased smoking (57). However, in our dataset, there were no mean differences in 8-OHdG between depressed smokers and nonsmokers and no interaction between smoking and depression on mean levels of 8-OHdG. Similarly, we were able to rule out exercise, alcohol consumption, BMI, medical illness, medication regimens, and other sociodemographic variables as potential mediating mechanisms contributing to group differences. We were not, however, able to rule out dietary differences between depressed and nondepressed individuals that could have potentially contributed to the group differences in 8-OHdG. The current study lacks measures of dietary variables and this question must remain unanswered for the time being.

Alternatively, it may be that depression contributes to oxidative damage through an increase in the production of, and exposure to, ROS. Depression is associated with an activation of innate immune responses (58), promotion of inflammation (59), and heightened vulnerability to latent infection (60,61). Activated phagocytes are significant sources of ROS and produce superoxide, hydrogen peroxide, nitric oxide, and peroxynitrite as part of the cytotoxic host response against invading pathogens (62). The ROS are antimicrobial; they damage lipid membranes and protein structures, thus destroying antigen-bearing cells. Oxidative damage is not limited to microbial targets, however, and extensive host tissue damage may result (e.g., [63]). ROS from activated phagocytes can damage DNA bases (64) and induce strand breaks in neighboring cells (65), leading some to argue that the hydroxyl radicals and peroxynitrite formed during inflammation are the greatest contributors to the oxidation of DNA (66). Measures of neutrophil activation and respiratory burst as well as cytokines such as IL-8, which activate neutrophils, may be a promising area of future research and could link studies of oxidative damage with the extensive literature on immune dysregulation in clinical depression.

Another possibility is that depression does not increase production or exposure to ROS but rather decreases repair of damaged DNA. There is some evidence that repair of x-ray-damaged DNA is slower among highly distressed psychiatric inpatients, the majority of whom were hospitalized for depression (67). Reductions in the fidelity or kinetics of repair could increase measured levels of oxidative damage products by allowing their persistence rather than increasing their formation. Unfortunately, the present research is unable to address these important questions, and future investigations may benefit by expanding the scope of study to include simultaneous measures of innate immune activation, DNA damage, and DNA repair.

This study has a number of limitations. From a technical standpoint, our assay coefficient of variation was marginally higher than is recommended (37) producing excess variability. However, the increased variability should have made it more difficult to detect group differences rather than less. Nonetheless, future studies should continually aim for more precise estimates of 8-OHdG. As already mentioned, we do not have measures of dietary habits or supplement use, particularly the consumption of dietary antioxidants. Previous research has demonstrated associations between oxidative damage and various dietary constituents (41,68–74), and these measures may provide additional insight into group differences in oxidative damage. Also, our cross-sectional design does not allow us to determine directions of causality, and we cannot definitively say that depression is causing increased oxidative damage. It may be that both depression and oxidative damage result from underlying cytokine responses to chronic inflammation (75–77). We also recognize that our depressed group represents an unusually "clean" sample in that they were medically healthy and currently

untreated for their depression. We additionally screened for the presence of comorbid acute infections and chronic disease, using these as exclusionary criteria. Although these selection biases may limit our generalizability, we feel that this was a real strength of our study because it removed the possibility of confounding by prevalent diseases, alternative disease processes or treatment regimens. Furthermore, we feel that this increased the efficiency of our study by minimizing misclassification bias and permitting a smaller sample size.

The clinical relevance of increased oxidative damage evidenced by clinically depressed individuals is currently unknown, and future studies should appropriately address this deficit. Despite this, our data are consistent with the hypothesis that oxidative damage to important macromolecules is a potential common pathophysiological mechanism underlying multiple comorbid conditions in depressed people. Future studies might include multiple oxidative damage markers to different macromolecules and should address whether depression remission remediates underlying oxidative damage.

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