Increased oxidative stress in patients with depression and its relationship to treatment

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Abstract

Oxidative stress may play a role in the pathogenesis of depression. We tested the hypothesis that urinary F_2 isoprostane, a robust marker of oxidative stress, was increased in patients with depression and associated with symptoms and response to treatment. Urinary F2 isoprostane was compared in 18 patients with depression and 36 age and sex matched control subjects. In patients, we tested the association between oxidative stress, depression questionnaires and antidepressant treatment. Urinary F_2 isoprostane excretion was significantly higher in patients with depression than in control subjects. This association remained significant after adjustment for age, sex and BMI. Depression symptom severity scores were not correlated with F_2 isoprostane excretion. Nine patients were treated with sertraline or bupropion for eight weeks. Depression severity rating scale scores decreased significantly and F_2 isoprostane excretion increased. The increase in F_2 isoprostane excretion was inversely correlated with the improvement in Hamilton Depression Rating 17 items. In conclusion, oxidative stress is increased in patients with depression. However, although treatment with either bupropion or sertraline reduces the symptoms of depression, it may increase F2 isoprostane excretion. These results suggest that alternative mechanisms, beyond oxidative stress, may be involved in the development of depression and subsequent responses to treatment.

Keywords

depression; oxidative stress; F_2 isoprostanes; antidepressants; sertraline; bupropion

1. Introduction

Depression is a heterogeneous clinical disorder and its cause is not clear. Several mechanisms have been implicated in its pathogenesis. These include genetic predisposition, deficiency of monoamines, abnormalities in circadian rhythm, hypercortisolemia, and increased inflammatory cytokines.(Belmaker and Agam, 2008) In addition, oxidative stress

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has been postulated to have an important role in the pathogenesis of depression.(Ng et al. 2008)

Oxidative stress is the imbalance between oxidant and antioxidants in favor of the oxidants, leading to a disruption of the redox signaling and control and/or molecular damage.(Sies and Jones D.P. 2007) There are several clinical conditions associated with increased oxidative stress, and different methods to assess it. The discovery of F_2 isoprostanes, products derived from lipid peroxidation, and the development of methods to quantify them accurately, have provided a robust marker of oxidative stress in vivo. (Morrow et al. 1999;Lawson et al. 1999;Cracowski et al. 2002; Nourooz-Zadeh et al. 2008)

Previous studies have linked increased measures of oxidative stress with affective disorders. Ozcan et al used superoxide dismutase (SOD) glutathione peroxidase (GSH-Px) and catalase (CAT) activities and malondialdehyde (MDA) and nitric oxide (NO) levels as measures of oxidative stress. Because enzymatic function was decreased and MDA levels increased in patients with affective disorders, the authors concluded that the antioxidant system was impaired during mood episodes. (Ozcan et al. 2004) Others have used different techniques, such lower total oxidant capacity and higher total oxidant status in patients with major depression.(Cumurcu et al. 2009) Furthermore, using an oxidative stress index, defined as the percent ratio of total peroxide plasma concentration to the total antioxidant potential, investigators have found an association between oxidative stress with higher Hamilton Depression Rating Scales. (Yanik et al. 2004) However, many of the techniques lack sensitivity and specificity to measure oxidative stress in vivo. Thus, we measured F2-isoprostanes, a technique recognized by experts as the most accurate method to evaluate oxidative stress. (Milne et al. 2007)

Despite the therapeutic effects of antidepressants, there are conflicting data regarding antidepressants and oxidative stress. Recent reports suggest that antidepressants may have antioxidant properties. For example, venlafaxine treatment produced anti-anxiety effects and attenuated oxidative damage (Kumar et al. 2010) and fluoxetine acted as an antioxidant in melanoma-induced oxidative changes. (Kirkova et al. 2010) However, because fluoxetine is a fluorinated compound, its hepatotoxicity is thought to be mediated by increased levels of oxidative stress (Inkielewicz-Stepniak ,2011) and pro- oxidant properties, through coenzyme Q deficiency, have been attributed to amitriptyline. (Bautista-Ferrufino et al. 2011)

Thus, we tested the hypothesis that oxidative stress, as determined by F_2 isoprostane excretion, was increased in patients with depression and was correlated with severity of symptoms and response to treatment.

2. METHODS

2.1. Study subjects

We studied 18 patients with major depressive episodes (MDE), diagnosed by the Structured Clinical Interview for DSM-IV (First et al. 1996) and confirmed by diagnostic interview with a senior psychiatrist (RMS). Patients were free of psychotropic use for at least 1 month prior to study, and at least 2 months for fluoxetine. Patients were older than 18 years of age and were recruited from newspaper and Vanderbilt community email announcements. Medical status was evaluated by physical examination and a routine laboratory screen, including pregnancy testing and urine drug screen. Any primary non-MDE comorbid diagnosis or substance abuse in the prior six months was cause for exclusion. Those who were currently using tobacco, had any sign of acute or chronic medical or neurological illness, or were pregnant were excluded from the study. Thirty-six non- smoking control subjects were selected by frequency-matching for age and sex from a cohort of control

subjects without inflammatory disease or depression. The study was approved by the Institutional Review Board of Vanderbilt University Hospital, and all subjects provided written informed consent.

2.2. F_2 isoprostanes

2,3-dinor-5,6-dihydro-15-F_2-Isoprostane, a urine metabolite and robust method of oxidative stress, was measured using gas chromatography/mass spectrometry as previously described. (Morrow and Roberts ,1994;Roberts and Morrow ,2000)

2.3. Questionnaires

All patients were interviewed for the Hamilton Depression Rating Scale, which has 17 items (Hamilton ,1967) and is widely used for the assessment of depression severity. Items are rated on a five point scale (0–4) except for items on weight loss, appetite, sleep disturbance, general somatic symptoms, loss of libido and insight that were rated on a three point scale (0–2).(Morriss et al. 2008) Patients were also evaluated with a modified version of the Hamilton Depression Rating Scale that includes 25 items.(Mazure et al. 1986) Higher scores in both instruments indicate higher levels of depression. Patients also completed the Profile of Mood States (POMS) instrument that includes 65 items. Each item is rated on a five-point scale. Scores are reported as global as well as six individual subscales (tension/anxiety, depression/dejection, anger/hostility, vigor, fatigue, and confusion).(Garland et al. 2007)

2.4. Treatment

A subgroup of nine patients with depression completed an open-label, random-assignment, 8 week treatment with either sertraline (n=6, 100 mg/day) or bupropion (n= 3, 300 mg mg/day). This subgroup of patients had a follow-up visit at week that included both Hamilton scores and POMS. Urine samples were collected before and after 8 weeks of therapy, frozen at –40 °C until isoprostane concentrations were measured.

2.5. Statistical analysis

Based on previous data showing that the mean F_2 isoprostane excretion in control subjects is 2.2 ng/mg creatinine with a standard deviation (SD) of 1.4,(Avalos et al. 2007) a post hoc calculation determined that a study with 18 patients with depression and 36 control subjects would have 92% power to detect a difference of 1.4 ng/mg creatinine in F_2 isoprostane excretion with a two-sided significance level of 5%. Given the variability between individuals in F_2 isoprostane excretion, a difference of 1 SD between control subjects and those with depression was considered likely to indicate a biologically important difference.

Baseline characteristics and outcome measures were compared between patients and controls using Wilcoxon rank-sum tests. The relationship between questionnaire scores and F_2 isoprostane excretion in patients with depression was evaluated using Spearman's rank correlation test. Pre and post-treatment results were analyzed with the use of Wilcoxon-sign rank test. All analyses used a 5% two sided level of significance and were performed using STATA 10.0 (STATA corp, Texas).

3. RESULTS

3.1. Patients

Patients and control subjects were of similar age (mean age 32.2±10.0 years and 32.3±7.6 years, respectively, p=0.78) and sex (67% female in both groups). Control subjects had higher BMI (27.0±4.8 kg/m^2) than depressed patients (25.2±7.3 kg/m^2, p=0.05). No patients or control subjects smoked.

3.2. F$_2$ isoprostanes and depression

Urinary F$_2$ isoprostane excretion was significantly higher in patients with depression [3.1 (1.9-4.8) ng/mg creatinine] than in control subjects [2.0 (1.3-2.5) ng/mg creatinine] (p=0.003). (Figure 1) A linear regression model with logarithmically transformed F$_2$ isoprostane concentrations as the dependent variable, and depression as independent variable, confirmed this association [Beta coefficient 0.52 (0.23-0.81), p=0.001], and it remained significant after adjustment for age and sex [Beta coefficient 0.52 (0.24-0.80), p<0.001], and after further adjustment for BMI [Beta coefficient 0.47 (0.19-0.75), p=0.001].

3.3. F$_2$ isoprostanes and symptoms of depression

At baseline, when depression scores were high, urinary F$_2$ isoprostanes were not significantly correlated with mood or depressive symptoms as reported by the POMS or the Hamilton scores respectively. (Table 1) In the 9 patients treated with antidepressants symptoms of depression and mood improved significantly. The median score for tension/anxiety decreased from 7 to 0, for depression/dejection from 35 to 4, for anger/hostility from 9 to 3, fatigue from 19 to 4, confusion from 10 to 2, and vitality increased from 4 to 8 (all p<0.05). The scores from both versions of the Hamilton also decreased significantly (p=0.01). (Table 2)

3.4. Use of antidepressants and F$_2$ isoprostanes

F$_2$ isoprostane excretion increased from 2.9 (1.8-4.2) to 4.9 (2.0-6.3) ng/mg creatinine (p=0.04), after treatment with either sertraline or bupropion. This trend was present for both sertraline [F$_2$ isoprostanes increased from 2.8 (1.7-3.3) to 4.3 (2.0-5.5) ng/mg creatinine] and bupropion [F$_2$ isoprostanes increased from 4.2 (1.8-7.3) to 6.3 (1.7-6.7) ng/mg creatinine].

Depression symptom scores and F$_2$ isoprostane excretion after treatment did not correlate (p all >0.15) except for a significant negative association between the POMS vitality score and F$_2$ isoprostane excretion (rho -0.83, p= 0.005), suggesting that patients who were more vital had low levels of oxidative stress. However, the increase in F$_2$ isoprostane excretion and the improvement in the 17 item Hamilton depression score between the baseline and after treatment visit were significantly correlated (rho=-0.76, p=0.02). (Figure 2)

4. DISCUSSION

The major findings of this study are that (1) oxidative stress as measured by F$_2$ isoprostane excretion is increased in patients with depression compared to age and sex matched control subjects, and (2) F$_2$ isoprostane excretion increases after effective treatment of depression.

Free radicals generated by oxidative stress result in lipid peroxidation and consequently tissue damage. This effect may be even more important in highly vulnerable organs such as the brain. The brain is vulnerable because of its high rate of oxygen use, its modest antioxidant defenses and because it is rich in lipids that provide substrate for lipid oxidation. (Ng et al. 2008)

Concordant with our finding of increased oxidative stress in patients with depression, a few studies have reported that patients with major depression have increased concentrations of malondialdehyde (Bilici et al. 2001;Sarandol et al. 2007) and 4-hydroxy-2-nonenal concentrations.(Selley,2004) However, these methods do not optimally measure the degree of oxidative stress present *in vivo.*

The discovery of F_2 isoprostanes, derived from free radical mediated lipid peroxidation, and the development of sensitive and specific mass spectrometry methods to measure them have provided a robust and sensitive marker of oxidative stress *in vivo*.(Morrow et al. 1999;Lawson et al. 1999;Cracowski et al. 2002) A recent study demonstrated that patients with depression had higher serum concentrations of F_2 isoprostanes.(Yager et al. 2010) However, serum concentrations of F2 isoprostanes may be affected by serum lipid concentrations with uncertainty regarding the need to further adjust for HDL, LDL, arachidonate or total lipid levels.(Halliwell and Lee ,2010) Thus, our study has used state-of-the-art methodology to measure oxidative stress in patients with depression.

In addition to demonstrating increased oxidative stress in patients with depression, our findings suggest that effective treatment with antidepressants is associated with increase rather than decrease oxidative stress. Consistent with our results, a preclinical study suggested an increase in oxidative stress after antidepressant exposure. Using rat glioma and human astrocytoma cell lines, Slamon found decreased concentrations of the antioxidant glutathione following toxic doses of antidepressants (Slamon and Pentreath ,2000) and suggested that oxidative stress may play a role in the cytotoxicity observed. Subsequently, other studies have shown decreases in markers of oxidative stress following antidepressant treatment.(Bilici et al. 2001;Kotan et al. 2011) Further study of dosage and time course effects may be indicated.

Although the number of patients we studied was small, the finding of increased isoprostanes excretion after treatment is interesting, and if confirmed, may suggest that oxidative stress is not causally related to the symptoms of depression, or alternatively, that there is a substantial time lag between symptom improvement and a decrease in oxidative stress.

Another potential explanation for the improvement in depression scores despite an increase in oxidative stress was that effective treatment for depression could result in another change associated with increased oxidative stress. For example, obesity is associated with increased F_2 isoprostane excretion, and it is possible that improvement of depression may have improved appetite and resulted in weight gain, although based on clinical observations this would be unlikely to occur over 8 weeks and does not appear to be a plausible explanation.

This study has some limitations. First, the sample size was small; though enough to test our hypothesis that levels of isoprostanes were increased in patients with depression. Second, markers of inflammation and metabolic variables beyond body mass index were not collected; therefore, we could not examine if the association of oxidative stress and depression was independent of inflammation.

Third, while some studies have shown increased markers of oxidative stress in peripheral blood of patients with depression; the association of oxidative stress measured directly from the central nervous system is less clear. With the goal to better understand this, Teyssier et al obtained postmortem prefrontal cortex from patients with depression and control subjects and measured gene expression of stress response, but did not find any associations.(Teyssier et al. 2011) Our study did not aim to look at markers of oxidative stress in tissue.

In conclusion, oxidative stress is increased in patients with depression but may not be causal since treatment with either bupropion or sertraline significantly reduced the symptoms of depression but increased F_2 isoprostane excretion.

Acknowledgments

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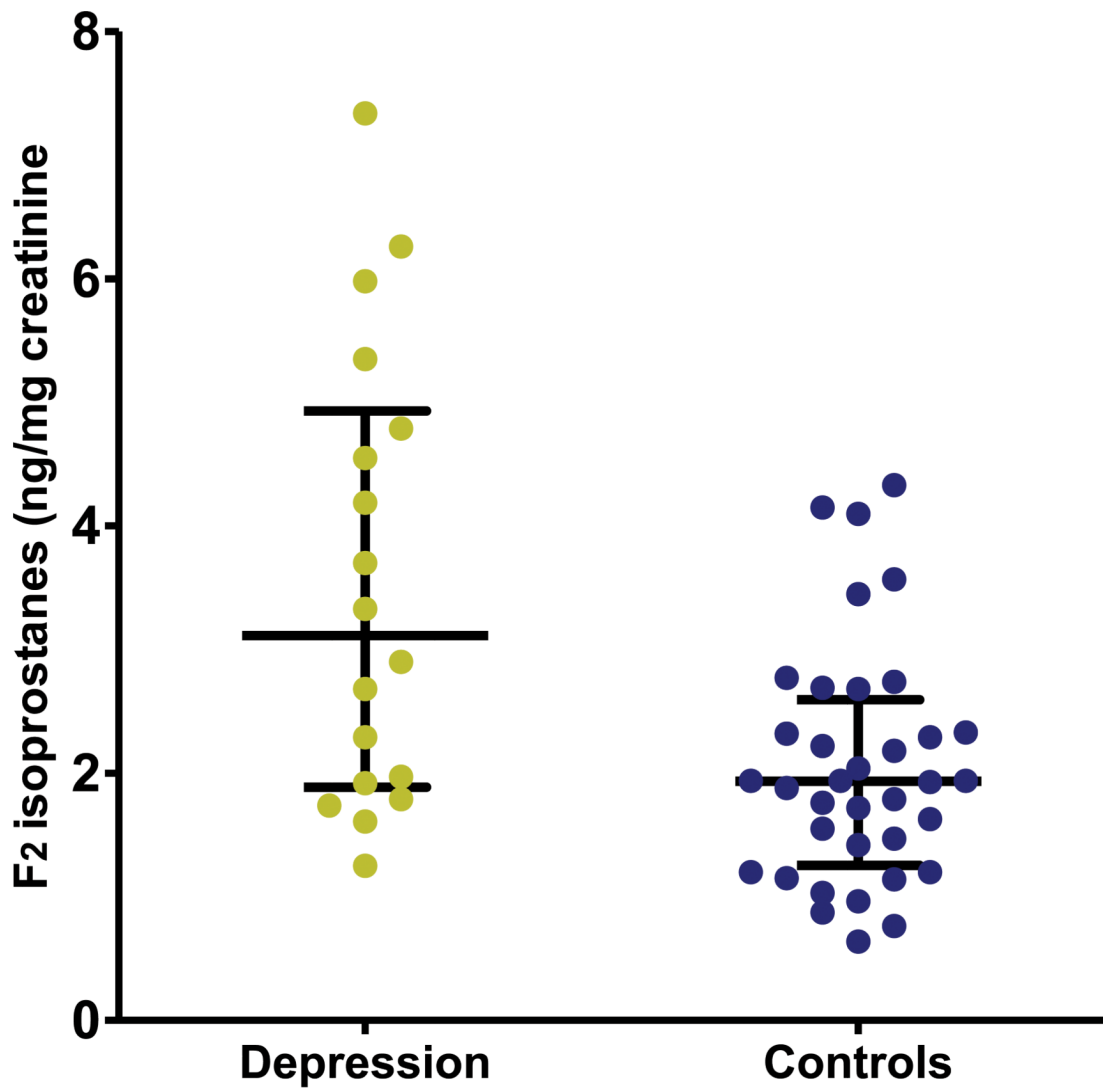
Lines represent median and interquartile range.
P=0.003

Figure 1.
Urinary F_2-isoprostane excretion in patients with depression and nes represent median and interquartile range P=0.003.
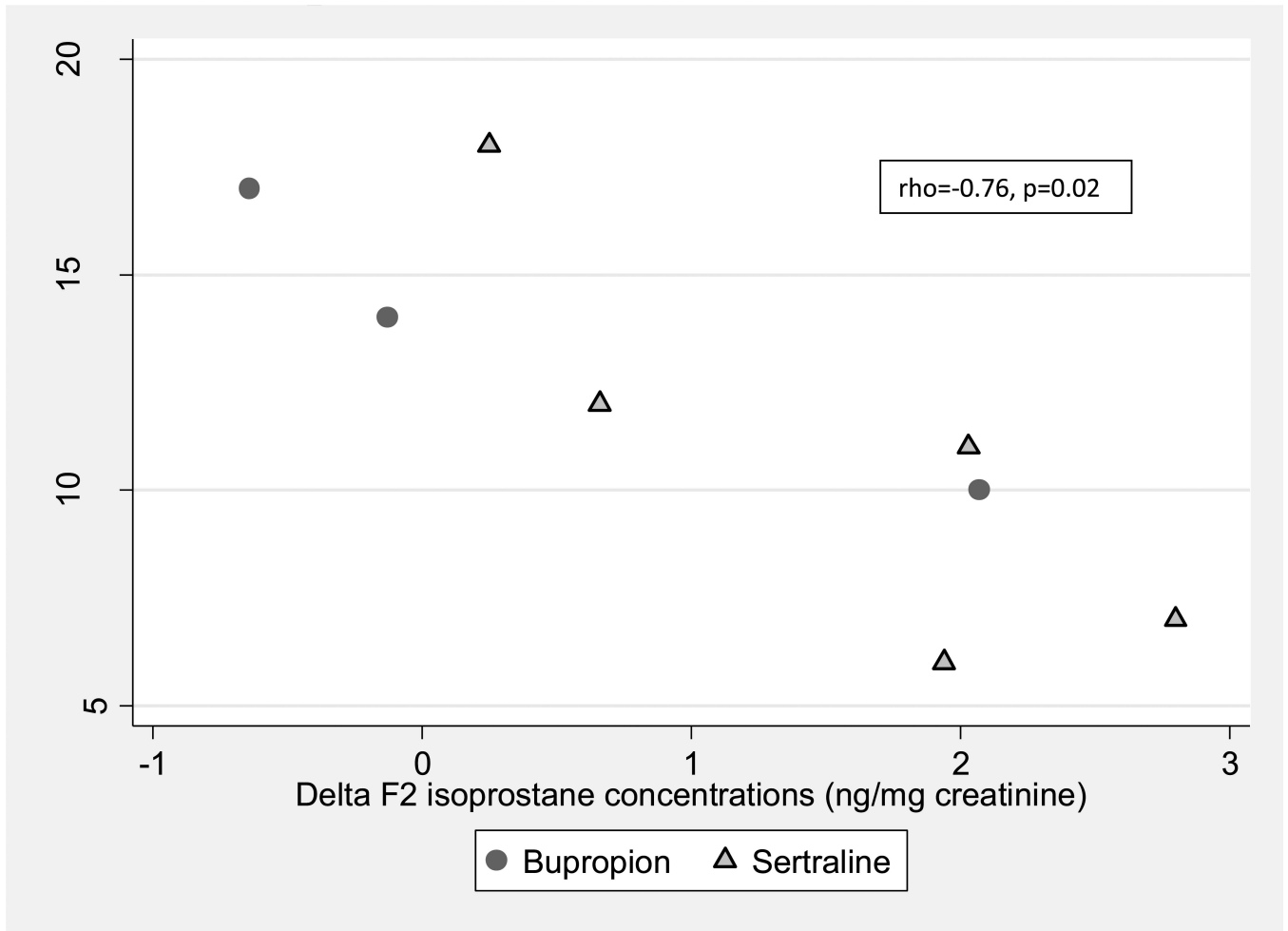
Figure 2.
Correlations between changes in the 17 item Hamilton score and changes in F_2isoprostanes after treatment with antidepressants

Table 1

Median [IQR] depression scale scores and their correlation with F_2 isoprostane excretion in patients with depression at baseline

Scale	Rho	P-value*
POMS-T	-0.09	0.70
POMS-D	0.07	0.78
POMS-A	-0.02	0.95
POMS-V	-0.20	0.43
POMS-F	-0.01	0.98
POMS-C	0.09	0.73
Hamilton 17 (n=9)	0.64	0.06
Hamilton 25 (n=9)	0.29	0.44

Table 2

Mood and depression scores in nine patients with depression at baseline and after treatment with antidepressants

Scale	Baseline (n=9)	After Treatment (n=9)	P-value[*]
POMS-T	7 (4 - 9)	0 (-1 – 2)	0.01
POMS-D	35 (33 – 17)	4 (1 - 18)	0.01
POMS-A	9 (2 – 16)	3 (0 – 3)	0.01
POMS-V	4 (3 - 5)	8 (6 – 12)	0.05
POMS-F	19 (7 - 22)	4 (2 - 6)	0.02
POMS-C	10 (8 - 12)	2 (1 – 7)	0.01
Hamilton 17	19 (18 – 23)	5 (2 – 8)	0.01
Hamilton 25	35 (26 – 38)	7 (5 – 15)	0.01

[*] Wilcoxon signed-rank test