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Major Differences in Neurooxidative and Neuronitrosative Stress Pathways Between Major Depressive Disorder and Types I and II Bipolar Disorder

Michael Maes [1,2,3,4] · Kamila Landucci Bonifacio [1] · Nayara Rampazzo Morelli [1] · Heber Odebrecht Vargas [1] · Décio Sabbatini Barbosa [1] · André F. Carvalho [5,6] · Sandra Odebrecht Vargas Nunes [1]

Abstract

Accumulating evidence indicates that oxidative and nitrosative stress (O&NS) pathways play a key role in the pathophysiology of bipolar disorder (BD) and major depressive disorder (MDD). However, only a handful of studies have directly compared alterations in O&NS pathways among patients with MDD and BD types I (BPI) and BPII. Thus, the current study compared superoxide dismutase (SOD1), lipid hydroperoxides (LOOH), catalase, nitric oxide metabolites (NOx), malondialdehyde (MDA), and advanced oxidation protein products (AOPP) between mood disorder patients in a clinically remitted state. To this end 45, 23, and 37 participants with BPI, BPII, and MDD, respectively, as well as 54 healthy controls (HCs) were recruited. Z-unit weighted composite scores were computed as indices of reactive oxygen species (ROS) production and nitro-oxidative stress driving lipid or protein oxidation. SOD1, NOx, and MDA were significantly higher in MDD than in the other three groups. AOPP was significantly higher in BPI than in HCs and BPII patients. BPII patients showed lower SOD1 compared to all other groups. Furthermore, MDD was characterized by increased indices of ROS and lipid hydroperoxide production compared to BPI and BPII groups. Indices of nitro-oxidative stress coupled with aldehyde production or protein oxidation were significantly different among the three patient groups (BDII > BDI > MDD). Finally, depressive symptom scores were significantly associated with higher LOOH and AOPP levels. In conclusion, depression is accompanied by increased ROS production, which is insufficiently dampened by catalase activity, thereby increasing nitro-oxidative damage to lipids and aldehyde production. Increased protein oxidation with formation of AOPP appeared to be hallmark of MDD and BPI. In addition, patients with BPII may have protection against the damaging effects of ROS including lipid peroxidation and aldehyde formation. This study suggests that biomarkers related to O&NS could aid in the differentiation of MDD, BPI, and BPII.

Keywords Depression · Bipolar disorder · Oxidative and nitrosative stress · Immune · Inflammation

✉ Michael Maes
dr.michaelmaes@hotmail.com; https://scholar.google.com.br/citations?user=1wzMZ7UAAAAJ&hl=pt-BR&oi=ao

Kamila Landucci Bonifacio
kamilalondrina@hotmail.com

Nayara Rampazzo Morelli
nayara.rampazzo@gmail.com

Heber Odebrecht Vargas
hebervargas@sercomtel.com.br

Décio Sabbatini Barbosa
sabbatini2011@hotmail.com

André F. Carvalho
andrefc7@hotmail.com

Sandra Odebrecht Vargas Nunes
sandranunes@sercomtel.com.br

[1] Health Sciences Graduation Program, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil

[2] Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[3] Department of Psychiatry, Medical University of Plovdiv, Plovdiv, Bulgaria

[4] IMPACT Strategic Research Centre, School of Medicine, Deakin University, PO Box 281, Geelong 3220, Vic, Australia

[5] Department of Psychiatry, University of Toronto, Toronto, ON, Canada

[6] Centre for Addiction & Mental Health, Toronto, ON, Canada

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Introduction

Research on the pathophysiology of major depressive disorder (MDD) and bipolar disorder (BD) has highlighted that aberrations in neurooxidative, neuronitrosative stress, and neuroimmune pathways play a key pathophysiological role in these disorders [1–7]. Chronic immune activation and inflammatory processes, as observed in mood disorders, are frequently accompanied by elevated levels of reactive oxygen (ROS) and nitrogen (RNS) species, including superoxide, peroxides, nitric oxide (NO), and peroxynitrite, while increased oxidative and nitrosative damage to lipids and proteins may cause immune activation [3, 5]. There are also data that point that activated neurooxidative, neuronitrosative, and neuroimmune pathways are interrelated phenomena in both major depression and bipolar disorder [3, 5, 8].

Both depression and BD are associated with lowered lipid-associated antioxidant defenses including lowered activity of lecithin cholesterol acyltransferase (LCAT), lower levels of high-density lipoprotein (HDL) cholesterol, vitamin E, coenzyme Q10 and paraoxonase 1 and glutathione peroxidase activities [9–17]. This specific reduction in lipid-targeted antioxidant defenses may contribute to increased ROS levels and oxidative damage to lipid membranes (lipid peroxidation) including to polyunsaturated fatty acids [18–20]. Lipid hydroperoxide chain reactions eventually cause the formation of reactive aldehydes, the end-product of lipid peroxidation, as indicated by increased levels of malondialdehyde (MDA) or thiobarbituric acid reactive substances (TBARS) and increased autoimmune responses (IgG- or IgM-mediated) directed against oxidatively formed neoepitopes, including azelaic acid and MDA, oxidized low-density lipoprotein cholesterol, and anchorage molecules [21–27]. Signs of lipid peroxidation coupled with reactive aldehyde production as measured with plasma TBARS or MDA are now among the most frequently reported biomarkers for depression and BD [21, 26–34]. Recent meta-analyses also report elevated TBARS and MDA concentrations in depression [35, 36] and BD [37, 38]. In both mood disorders, increased TBARS seem to be associated with severity of illness, suicidal behaviors, and the number of manic and/or depressive episodes in the year prior to the assay of MDA [8]. No significant differences could be detected in MDA levels and associated immune-inflammatory biomarkers among patients in acute phases of depression versus BD [8, 39]. Therefore, it remains unclear whether specific aspects of the nitro-oxidative pathways ranging from ROS production to lipid peroxidation could differ between individuals with depression and BD. These abnormalities may include changes in superoxide dismutase (SOD) activity, lipid hydroperoxide levels (LOOH), catalase activity, increased ROS coupled with RNS, or lipid peroxidation with aldehyde formation.

Major depression is accompanied not only by increased ROS and lipid peroxidation but also by oxidative damage to proteins as indicated by elevated levels of advanced oxidation protein products (AOPPs) [40, 41]. AOPPs are formed via increased ROS and peroxynitrite production coupled with increased myeloperoxidase activity and hypochlorous acid production [42]. Depression is also characterized by increased inducible nitric oxide (NO) synthase (iNOS) activity and NO production, which eventually may lead to nitrosative stress and hypernitrosylation [2, 3, 43, 44]. In addition, evidence suggests that NO production is elevated in euthymic patients with BD compared to controls [45]. Therefore, major affective disorders are now conceptualized as neurooxidative, neuronitrosative, and neuroimmune disorders, which are characterized by nitro-oxidative and nitrosative stress (O&NS)-induced neurotoxic responses leading to aberrations in neuroprotection, neuronal functions, neurogenesis, synaptic plasticity, neurotransmitter signaling, and receptor expression [3, 4, 46]. Potential differences between depression and BD on specific aspects of O&NS pathways remain under-explored.

Therefore, the aim of the present study was to examine levels of SOD, LOOH, catalase, NO metabolites (NOx), MDA, and AOPP among clinically stable patients with depression, type I BD (BDI), and BPII, as well as healthy controls (HCs). The a priori hypothesis was that these mood disorders are accompanied by activated O&NS pathways and that are no significant differences between depression and BD would emerge.

Subjects and Methods

Participants

In this cross-sectional study, we included 54 HCs and 105 patients with mood disorders, namely 37 patients with MDD, 45 with BPI, and 23 with BPII. All participants were Brazilian of both gender and aged 20 to 63 years old. All participants with mood disorders were outpatients admitted to the Psychiatry outpatient clinics at the University Hospital of the Universidade Estadual de Londrina (UEL), Parana, Brazil. They were all in remission or partial remission, and the index episode in BD patients was not of (hypo)manic polarity. The HC sample was derived from the same catchment area. The following exclusion criteria were applied for patients and controls: (a) pregnant women; (b) subjects with medical illness affecting immune functions, including hepatitis B and C virus infection, HIV infection, neuroimmune and neurodegenerative disorders (e.g., Alzheimer's disease, multiple sclerosis, Parkinson's disease), chronic obstructive pulmonary disease, chronic kidney disease, cancers, autoimmune diseases such as rheumatoid arthritis, type 1 diabetes, and systemic lupus erythematosus; (c) subjects with other axis-1

diagnoses according to DSM-IV-TR criteria, including schizophrenia, schizo-affective disorder, autism, psycho-organic syndromes; and (d) subjects who were treated with nonsteroidal anti-inflammatory drugs, interferon, glucocorticoids, antioxidants, herbal supplements and omega-3 polyunsaturated fatty acids during the past 4 weeks prior to study enrollment. Some patients with MDD and BD were currently treated with antidepressants ($n = 44$), atypical antipsychotics ($n = 32$), lithium ($n = 26$), and other mood stabilizers ($n = 33$) including carbamazepine and valproic acid. All participants provided written informed consent to take part in the current study, whose experimental procedures were previously approved by the Research Ethics Committee at UEL (protocol number CAAE 34935814.2.0000.5231).

Methods

The clinical diagnoses of MDD, BPI, and BPII were made by a research psychiatrist using the validated Brazilian Portuguese version of the structured clinical interview for DSM-IV interview (SCID) axis I [47] in accordance with *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision* (DSM-IV-TR) diagnostic criteria [48]. Moreover, all participants completed a semi-structured interview comprising socio-demographic data (self-perceived ethnicity, years of education, marital status) and clinical data (number of previous depressive, hypomanic, and manic episodes). We used the 17-item Hamilton Depression Rating Scale (HAM-D), translated and adapted for use with Brazilian individuals [49], to measure severity of depression, while severity of manic symptoms was scored employing the Brazilian Portuguese version of the Young Mania Rating Scale (YMRS) [50]. The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) was employed to assess substance misuse, namely use of alcohol and hypnotics. This rating scale was developed by the World Health Organization [51] and translated into Brazilian Portuguese by Henrique et al. [52]. The diagnosis of nicotine dependence was made with the Fagerstrom Nicotine Dependence Scale [53], which has been previously validated for use in Brazilian samples [54]. We used two cutoff values yielding three groups, namely 0–1, no nicotine dependence; 2–5, mild dependence; and ≥ 6, severe dependence.

A diagnosis of metabolic syndrome (MetS) was made according to the International Diabetes Federation criteria [55], namely presence of three out of the following criteria: (a) abdominal obesity (waist circumference ≥ 90 cm for men and ≥ 80 cm for women in South Asian and South Americans and ≥ 94.0 cm for men and ≥ 80.0 cm for women in Caucasians); (b) low HDL cholesterol (< 40 mg/dL in men and < 50 mg/dL in women) or use of hypolipidemic drugs; (c) hypertriglyceridemia (triglycerides > 150 mg/dL) or use of hypolipidemic agent; (d) increased fasting glucose (> 100 mg/dL) or use of oral antidiabetic medications; and (e) increased average blood pressure (130/85 mmHg) or currently taking antihypertensive medication. We measured the body mass index (BMI) according to the following formula: weight (in kg) divided by square of height (in m^2).

Assays

Peripheral fasting (12 h) blood was sampled at 8 a.m. the same day as the diagnosis was made and clinical data were collected. We measured the activities of superoxide dismutase (SOD1) and catalase and the concentrations of lipid hydroperoxides (LOOH), NO metabolites (NOx), malondialdehyde (MDA) and advanced oxidation protein products (AOPP). SOD activity in erythrocytes was determined using the pyrogallol method described by Marklund and Marklund [56]. This technique is based on the inhibition of pyrogallol self-oxidation by SOD in aqueous solution. The assay was conducted in a spectrophotometer Helios α, Thermo Spectronic (Waltham, MA, USA) at 420 nm and 37 °C. During 5 min, variation in optical density (OD) was recorded every minute. The level of SOD that inhibited 50% of the pyrogallol oxidation was defined as one unit of enzymatic activity. The results were expressed U/mg of hemoglobin (Hb). Lipid hydroperoxides (LOOH) are assayed by chemiluminescence (CL-LOOH) [57, 58]. This method uses the compound tert-butyl hydroperoxide to start a lipid chain reaction that can be detected by photon emission during the formation of lipid hydroperoxides. Readings were performed in a Glomax luminometer (TD 20/20 Turner Designers, USA) over 1 h at one reading per second. Results are expressed as relative units of light. Measurement of catalase activity was estimated through the difference between the initial reading and the reading conducted 30 s after the addition of 200 mM H_2O_2 30% at 240 nm in a microplate reader (model EnSpire, Perkin Elmer, USA) with the temperature maintained at 25 °C. The catalase values are expressed as U/mg Hb. NO metabolite (NOx) levels were assessed indirectly by determining the plasma nitrite concentration using an adaptation of the technique described Navarro-Gonzalvez et al. [59]. This method is based on the reduction of the nitrate present in the sample to nitrite by oxidation-reduction reactions mediated by the system cadmium-copper reagent. Thereafter, Griess reagent was added to induce diazotization, forming a colored complex and subsequent detection at 540 nm. The quantification of NOx was made in a microplate reader Asys Expert Plus, Biochrom (Holliston, MA, USA). The nitric oxide concentration was expressed in μM. MDA levels were measured through complexation with two molecules of thiobarbituric acid (TBA) using MDA estimation through high performance liquid chromatography (HPLC Alliance e2695, Waters', Barueri, SP, Brasil) [60]. Experimental conditions included the use of a column Eclipse XDB-C18 (Agilent, USA), mobile phase

consisting of 65% phosphate buffer (50 nM pH 7.0) and 35% HPLC-grade methanol, flow rate of 1.0 mL/min, temperature of 30 °C, and wavelength of 532 nm. MDA concentration in the samples was quantified based on a calibration curve and are expressed in mmol of MDA/mg proteins. AOPP was quantified using the method described by Hanasand et al. [61] in a microplate reader, Perkin Elmer, model EnSpire (Waltham, MA, EUA), at a wavelength of 340 nm. AOPP concentration was expressed in μM of equivalent chloramine T.

Figure 1 shows the pathway from superoxide formation to lipid peroxidation and the generation of aldehydes. In order to examine this pathway and the pathway to AOPP formation, we computed five composite scores reflecting different O&NS concepts.

1. zLOOH+SOD1 computed as z value LOOH (zLOOH) + zSOD. Increased superoxide induces SOD thereby catalyzing superoxide radicals into peroxides [62]. Lipid hydroperoxides are mainly derived from cholesterol, unsaturated phospholipids, and glycolipids and are intermediates of peroxidative reactions, which may be induced by hydroxyl radicals, peroxides, peroxyl radicals, and peroxynitrite [63]. As such, the sum of zLOOH + zSOD reflects ROS (peroxide + hydroxyl) production leading to lipid peroxidation.

2. zLOOH+SOD-CAT computed as zLOOH + zSOD − zCAT. Catalase catalyzes the decomposition of peroxides into oxygen and water, and therefore, this composite score reflects the formation of ROS (peroxide + hydroxyl) production leading to lipid peroxidation taking into account the protective effects of catalase.

3. zLOOH+SOD+NOx computed as zLOOH + zSOD + zNOx. This score reflects ROS (peroxide + hydroxyl) production leading to lipid peroxidation coupled with the production of NO and is therefore an index of nitro-oxidative stress and the potential to generate peroxynitrite and lipid hydroperxides [63, 64].

4. zLOOH+SOD+NOx+MDA computed as zLOOH + zSOD + zNOx + zMDA reflects the pathway from ROS production to nitro-oxidative stress and lipid peroxidation leading to increased production of reactive aldehydes with long-lasting detrimental consequences [3].

zLOOH+SOD+NOx+AOPP computed as zLOOH + zSOD + zNOx + zAOPP. This composite score reflects increased nitro-oxidative stress leading to protein oxidation (AOPP) via increased ROS and peroxynitrite production coupled with increased myeloperoxidase activity and hypochlorous acid production [42].
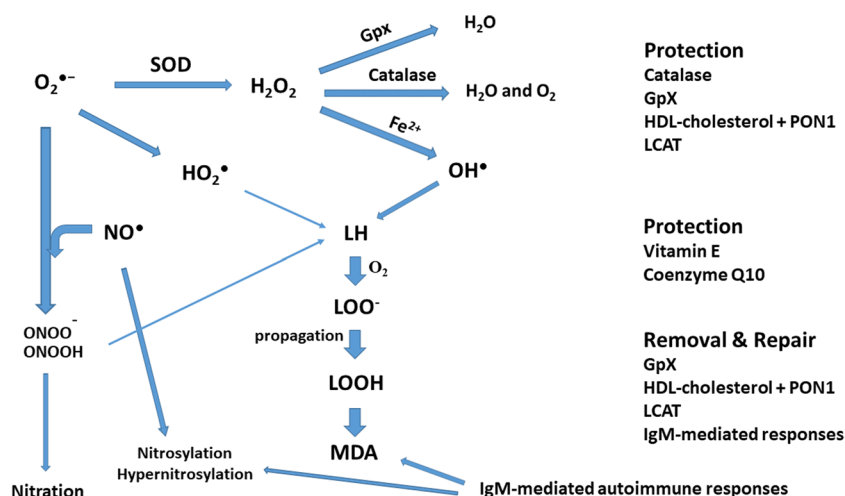

Fig. 1 Increased superoxide ($O_2^{\bullet-}$) is accompanied by increased superoxide dismutase (SOD), which catalyzes superoxide radicals into peroxides. Catalase and glutathione peroxidase (Gpx) catalyze the decomposition of peroxides into oxygen and water. The Fenton reaction (iron and hydrogen peroxides) may generate superoxide-dependent hydroxyl radicals ($OH^{\bullet}$) which together with perhydroxyl radicals ($HO_2^{\bullet}$) and peroxynitrite ($ONOO^-$) attack lipid membranes especially polyunsaturated fatty acids (PUFAs). The generated fatty acid radicals react with oxygen and form lipid peroxy (LOO^-) radicals which react with other lipids or PUFAs producing lipid hydroperoxides (LOOH), thereby damaging the lipid membranes and causing formation of aldehydes, including malondialdehyde (MDA), which are the end-product of lipid peroxidation. Different antioxidant defenses may protect against lipid peroxidation, including Gpx, catalase, high-density lipoprotein (HDL) cholesterol, paraoxonase 1 (PON1) and L-lecithin/cholesterol acyltransferase (LCAT), and cell membrane-associated vitamin E and coenzyme Q10. Removal (excision) and repair of the lipid peroxidation lesions may protect against lipid peroxidation-induced cell apoptosis or necrosis, including Gpx, HDL cholesterol, LCAT, and natural IgM-mediated autoimmune responses directed to MDA. Increased levels of nitric oxide (NO) react with superoxide to form peroxynitrous acid (ONOOH) and peroxynitrite, which consequently may contribute to lipid peroxidation and cause oxidation and nitration of proteins. Increased NO production may lead to nitrosylation or hypernitrosylation of proteins. An IgM-mediated autoimmune response may be mounted against neoepitopes, including NO (nitroso)- and NO2 (nitro)-adducts and MDA

Statistical Analyses

Differences in scale variables between diagnostic groups were assessed using analyses of variance (ANOVAs), while differences in nominal variables between diagnostic groups were assessed using analyses of contingency tables (χ^2 tests). Multivariate GLM analysis with the six O&NS biomarkers or the five z-unit composite scores as dependent variables were used to assess the effects of diagnosis (primary explanatory variable), while adjusting for age, sex, BMI, years of education, and nicotine dependence. Tests for between-subject effects were used to delineate the effects of the primary explanatory variable on the separate biomarkers and composite scores. Model-derived estimated marginal means were computed, and post hoc analyses were used to assess the differences between the diagnostic categories. Linear multiple regression analyses were employed to delineate the associations between one dependent variable (the biomarkers) and a set of explanatory variables. Binary logistic regression analysis was used to delineate the most significant predictors of diagnostic groups. Nagelkerke values are used as effect estimate, and odds ratios and 95% confidence intervals are computed. Results of multiple comparisons were p-corrected for false discovery rate according to Benjamini and Hochberg [65]. We used the IBM SPSS Windows version 22 and Statistica 8 to analyze all data. Statistical significance was set at 0.05, two-tailed.

Results

Descriptive Statistics

Table 1 shows the socio-demographic and clinical data in normal volunteers and patients with mood disorders. There were no significant differences in age, sex, marital status, BMI, MetS, and Fagerstrom score between the two groups. After p-correction for false discovery rate, there were no significant differences in education ($p = 0.085$), ethnicity ($p = 0.113$), and YMRS ($p = 0.115$) between both samples. The HAM-D score was higher in patients with mood disorders as compared with controls (p-correction: $p = 0.017$). There were no significant differences in the raw O&NS biomarkers between both groups (unadjusted for confounders such as age, sex, education, BMI, nicotine dependence). The correlation matrix among the six biomarkers shows that (without p-correction) there are significant correlations between SOD1 and MDA ($r = 0.310$, $p < 0.001$, $n = 136$) and between LOOH and AOPP ($r = 0.352$, $p < 0.001$, $n = 137$). All other correlation coefficients were non-significant.

Differences Between the Four Study Groups

Figure 1 shows the z-transformed values of the six O&NS biomarkers in the four study groups. Table 2, regression #1, shows the results of multivariate GLM analysis with the six biomarkers as dependent variables and diagnosis as primary explanatory variable, while adjusting for age, sex, education, nicotine dependence, and BMI. We found a significant effect of diagnosis on the six biomarkers (for sex and education: see below). Tests for between-subject effects showed significant effects of diagnosis on SOD1 and AOPP levels. Table 3 shows the model-generated estimated marginal mean values after adjusting for the confounders. Post hoc analyses showed that SOD1 was significantly higher in MDD patients as compared with controls and BPII patients, while those with BPI showed an intermediate position. AOPP levels were significantly higher in BPI than in controls and BPII patients, while MDD patients occupied an intermediate position.

Figures 2 and 3 show the z-transformed values of the O&NS biomarkers and the five composite scores in the four study groups, respectively. Table 2, regression #2, shows the results of a multivariate GLM analysis with the five composite scores as dependent variables and diagnostic groups as primary explanatory variables, while adjusting for age, sex, education, nicotine dependence, and BMI. We found a significant effect of diagnostic groups on the five composite scores. Tests for between-subject effects showed significant effects on all scores. Table 3 shows that the five scores were significantly higher in major depression than in controls (except LOOH+ SOD-CAT) and patients with BPII. Moreover, zLOOH+SOD, zLOOH+SOD+NOx, and zLOOH+SOD+NOx+MDA were significantly higher in major depression than in BPI. zLOOH+SOD and zLOOH+SOD+NOx+AOPP were significantly higher in BPI than BPII, while LOOH+SOD+NOx+ MDA was significantly lower in BPI than in controls.

Effects of the HAM-D Score

In order to adjust the effects of diagnosis for severity of illness, we have entered the HAM-D score in regression #2, Table 2. The results of this analysis (Table 2, regression #3) show that diagnosis and HAM-D were both significant. Nevertheless, the HAM-D was only associated with zLOOH+SOD+NOx+ AOPP, while the effects of diagnosis on all five composite scores remained significant. After adding the YMRS score in regression #2, Table 2, no significant effects of YMRS on the composite scores was found ($F = 0.93$, $df = 5/113$, $p = 0.462$).

We have also examined the differences among the three diagnostic groups after entering the dichotomized HAM-D score (cutoff value < 7 versus ≥ 7) as a second factor, thereby adjusting for the remitted versus the non-remitted state (while also adjusting for sex, age, education, nicotine dependence,

Table 1 Socio-demographic, clinical, and biomarker data of patients with mood disorders (MOOD) and healthy controls (HCs)

Variables	HC ($n = 54$)	MOOD ($n = 105$)	F/X^2	df	p
Age (years)	43.6 (11.7)	42.7 (10.8)	0.28	1/157	0.598
Sex (M/F)	44/23	30/7	5.09	2	0.078
Education (years)	12.5 (5.8)	10.3 (4.7)	6.76	1/157	0.010
Single/separated-widowed /married	15/10/ 29	22/26/57	1.33	2	0.514
Caucasian/other	34/20	84/21	5.41	1	0.020
Body mass index (kg/m^2)	26.3 (4.9)	26.6 (5.0)	0.09	1/149	0.092
Metabolic syndrome (no/yes)	33/20	66/39	0.00	1	0.942
Fagerstrom score	2.8 (3.3)	3.1 (3.4)	0.56	1/157	0.454
Fagerstrom_3 groups[a]	30/7/17	53/18/34	0.58	2	0.749
HAM-D [q25 – q75]	2.6 (3.4) [0–4]	9.9 (6.5) [4–13]	58.95	1/157	< 0.001
YMRS	0.8 (1.7)	1.7 (2.5)	4.99	1/157	0.027
Number depressive episodes	–	5.1 (4.6)	–	–	–
Number manic episodes	–	4.4 (6.2)	–	–	–
SOD1 (U/mg Hb)	91.4 (40.8)	101.0 (41.7)	1.67	1/134	0.199
LOOH (RLU × 10^6)[b]	1552 (1011)	1640 (1149)	0.27	1/135	0.607
CAT (U/mg Hb)	57.1 (14.9)	62.0 (14.4)	3.56	1/133	0.061
NOx (μM)[b]	6.1 (3.2)	6.9 (3.8)	1.34	1/136	0.249
MDA (mmol/mg of protein)[b]	64.3 (22.1)	65.6 (22.3)	0.12	1/136	0.731
AOPP (μM)[b]	71.6 (44.6)	85.5 (43.3)	2.72	1/136	0.076

All results are shown as mean (± SD)

F results of analyses of variance, X^2 results of analyses of contingency tables, *HAM-D* Hamilton Depression rating Scale score, *Q25 and q75* 25% and 75% quartile values, *SOD* superoxide dismutase, *LOOH* lipid hydroperoxides, *CAT* catalase, *NOx* nitric oxide metabolites, *MDA* malondialdehyde, *AOPP* advanced oxidation protein products

[a] Three groups using cutoff values 2 and 6 (thus group 1, 0–1; group 2, 3–5; group 3, ≥ 6)

[b] These data are processed in Ln transformation

and BMI). Multivariate GLM analysis #4, Table 2, shows a significant effect of diagnosis, but not of the dichotomized HAM-D scores. Figure 4 shows the residualized composite scores after regression on age, sex, BMI, education, nicotine dependence, and the dichotomized HAM-D values in the three mood disorders groups. zLOOH+SOD and zLOOH+SOD-CAT were significantly higher in major depression that in BPI and BPII, while there were no differences between both BD subtypes ($p = 0.066$ and $p = 0.166$, respectively). zLOOH+SOD+NOx, zLOOH+SOD+NOx+MDA, and zLOOH+SOD+NOx+AOPP were significantly different between the three groups and increased from BPII to BPI to major depression.

In multivariate GLM analysis #5, Table 2, we examined the differences between the three mood disorder groups in subjects with a HAM-D score < 11 (14 BP1, 13 BP2, and 22 depressed patients). Also, this analysis showed a significant effect of diagnostic groups with an effect size of 0.306 and univariate effects on all five scores. Figure 5 shows the unadjusted mean values of the composite scores in the three study groups with HAM-D values < 11. zLOOH+SOD, zLOOH+SOD-CAT, zLOOH+SOD+NOx, and zLOOH+SOD+NOx+AOPP were significantly higher in depression and BPI than

BPII patients, while there were no significant differences between depressed and BPI patients. zLOOH+SOD+NOx+MDA was significantly different between the three groups and increased from BPII to PBI to depression.

Effects of Confounding Variables

LOOH ($F = 5.46$, $df = 1/118$, $p = 0.021$; partial eta squared = 0.044) and AOPP ($F = 23.52$, $df = 1/118$, $p < 0.001$, partial eta squared = 0.166) were significantly higher in males than females. All five composite scores were significantly higher in men than in women. Education was significantly and inversely associated with SOD1 ($F = 6.91$, $df = 1/118$, $p = 0.010$, partial eta squared = 0.055), MDA ($F = 8.90$, $df = 1/118$, $p = 0.003$, partial eta squared = 0.070), zLOOH+SOD ($F = 4.71$, $df = 1/118$, $p = 0.032$, partial eta squared = 0.038), zLOOH+SOD-CAT ($F = 5.83$, $df = 1/118$, $p = 0.017$, partial eta squared = 0.047), and zLOOH+SOD+NOx+MDA ($F = 5.65$, $df = 1/118$, $p = 0.019$, partial eta squared = 0.046). There were no significant effects of age, nicotine dependence, and BMI on the biomarkers.

In order to examine possible effects of other confounders on the associations between diagnostic groups and the five

Table 2 Results of multivariate general linear model (GLM) analysis with six oxidative and nitrosative stress biomarkers as dependent variables and diagnostic groups, namely controls (HC), bipolar 1 (BPI), bipolar 2 (BPII), and major depression (MDD) as primary explanatory variables, while adjusting for age, sex, body mass index (BMI), nicotine dependence (ND), and education

Type test	Dependent variables	Explanatory variables	F	df	p	Partial eta squared
Multivariate ≠ 1	SOD1, LOOH, CAT, NOx, MDA, AOPP	HC, BPI, BPII, MDD	2.20	18/320	0.004	0.104
		ND_3groups	1.64	12/226	0.078	0.081
		Age	1.17	6/113	0.328	0.058
		Sex	4.98	6/113	< 0.001	0.209
		BMI	1.46	6/113	0.197	0.072
		Education	2.90	6/133	0.011	0.133
Between-subject effects	SOD1	HC, BPI, BPII, MDD	3.95	3/118	0.010	0.091
	LOOH	HC, BPI, BPII, MDD	1.97	3/118	0.178	0.041
	CAT	HC, BPI, BPII, MDD	0.77	3/118	0.516	0.019
	NOx	HC, BPI, BPII, MDD	2.38	3/118	0.083	0.055
	MDA	HC, BPI, BPII, MDD	1.02	3/118	0.296	0.031
	AOPP	HC, BPI, BPII, MDD	3.05	3/118	0.031	0.072
Multivariate #2	zLOOH+SOD,	HC, BPI, BPII, MDD	2.49	15/315	0.002	0.095
	zLOOH+SOD-CAT,	ND_3groups	1.54	10/228	0.126	0.063
	zLOOH+SOD+NOx,	Age	0.79	5/114	0.558	0.034
	zLOOH+SOD+NOx+MDA,	Sex	6.02	5/114	< 0.001	0.209
	zLOOH+SOD+NOx+AOPP	BMI	1.71	5/114	0.137	0.070
		Education	3.37	5/114	0.007	0.129
Between-subject effects	zLOOH+SOD	HC, BPI, BPII, MDD	5.21	3/118	0.002	0.117
	zLOOH+SOD-CAT	HC, BPI, BPII, MDD	2.88	3/118	0.039	0.068
	zLOOH+SOD+NOx	HC, BPI, BPII, MDD	7.35	3/118	< 0.001	0.157
	zLOOH+SOD+NOx+MDA	HC, BPI, BPII, MDD	8.18	3/118	< 0.001	0.172
	zLOOH+SOD+NOx+AOPP	HC, BPI, BPII, MDD	7.35	3/118	< 0.001	0.157
Multivariate #3[a]	zLOOH+SOD,	HC, BPI, BPII, MDD	1.99	15/312	0.016	0.080
	zLOOH+SOD-CAT,	HAM-D	3.30	5/113	0.008	0.127
	zLOOH+SOD+NOx,					
	zLOOH+SOD+NOx+MDA,					
	zLOOH+SOD+NOx+AOPP					
Between-subject effects	zLOOH+SOD	HC, BPI, BPII, MDD	5.53	3/117	0.001	0.124
	zLOOH+SOD-CAT	HC, BPI, BPII, MDD	3.27		0.024	0.077
	zLOOH+SOD+NOx	HC, BPI, BPII, MDD	7.25		< 0.001	0.157
	zLOOH+SOD+NOx+MDA	HC, BPI, BPII, MDD	8.29		< 0.001	0.175
	zLOOH+SOD+NOx+AOPP	HC, BPI, BPII, MDD	7.08		< 0.001	0.148
	zLOOH+SOD+NOx+AOPP	HAM-D	7.08	1/117	< 0.001	0.057
Multivariate #4[a]	zLOOH+SOD,	BPI, BPII, MDD	3.48	10/134	< 0.001	0.206
	zLOOH+SOD-CAT,	Dichotomized HAM-D	1.05	5/67	0.398	0.072
	zLOOH+SOD+NOx,	(< 7 versus ≥ 7)				
	zLOOH+SOD+NOx+MDA,					
	zLOOH+SOD+NOx+AOPP					
Between-subject effects	zLOOH+SOD	BPI, BPII, MDD	9.07	2/71	< 0.001	0.204
	zLOOH+SOD-CAT	BPI, BPII, MDD	6.96	2/71	0.002	0.164
	zLOOH+SOD+NOx	BPI, BPII, MDD	10.46	2/71	< 0.001	0.228
	zLOOH+SOD+NOx+MDA	BPI, BPII, MDD	15.25	2/71	< 0.001	0.301
	zLOOH+SOD+NOx+AOPP	BPI, BPII, MDD	11.73	2/71	< 0.001	0.248
Multivariate #5[a]	zLOOH+SOD,	BPI, BPII, MDD in patients	3.17	10/74	0.003	0.306
	zLOOH+SOD-CAT,	with HAM-D < 11 only				
	zLOOH+SOD+NOx,					
	zLOOH+SOD+NOx+MDA,					
	zLOOH+SOD+NOx+AOPP					
Between-subject effects	zLOOH+SOD	BPI, BPII, MDD	4.35	2/40	0.020	0.179
	zLOOH+SOD-CAT	BPI, BPII, MDD	6.07	2/40	0.005	0.233
	zLOOH+SOD+NOx	BPI, BPII, MDD	5.86	2/40	0.006	0.227
	zLOOH+SOD+NOx+MDA	BPI, BPII, MDD	8.02	2/40	0.001	0.286
	zLOOH+SOD+NOx+AOPP	BPI, BPII, MDD	8.84	2/40	0.001	0.306

zLOOH+SOD computed as z transformation of LOOH (zLOOH) + zSOD

zLOOH+SOD-CAT computed as zLOOH + zSOD − zCAT

zLOOH+SOD+NOx computed as zLOOH + zSOD + zNOx

zLOOH+SOD+NOx+MDA computed as zLOOH + zSOD + zNOx + zMDA

zLOOH+SOD+NOx+AOPP computed as zLOOH + zSOD + zNOx + zAOPP

SOD superoxide dismutase, *LOOH* lipid hydroperoxides, *CAT* catalase, *NOx* nitric oxide metabolites, *MDA* malondialdehyde, *AOPP* advanced oxidation protein products

[a] All multivariate and univariate GLM analyses are adjusted for age, sex, nicotine dependence (ND), years of education and BMI

Table 3 Model-generated estimated marginal mean (SE) values (expressed in z values) obtained by the general linear model analyses shown in Table 2

Variables	Healthy controls[A]	BPI[B]	BPII[C]	MDD[D]
SOD1	− 0.09 (0.15)[D]	+ 0.04 (0.20)	− 0.52 (0.23)[D]	+ 0.46 (0.18)[A,C]
LOOH	+ 0.19 (0.15)	+ 0.16 (0.20)	− 0.06 (0.23)	+ 0.57 (0.18)
CAT	− 0.36 (0.16)	− 0.06 (0.22)	− 0.17 (0.25)	− 0.05 (0.20)
NOx	− 0.24 (0.17)	− 0.16 (0.22)	− 0.28 (0.26)	+ 0.39 (0.20)
MDA	+ 0.23 (0.15)	+ 0.10 (0.20)	− 0.10 (0.23)	+ 0.44 (0.18)
AOPP	+ 0.04 (0.13)[B]	+ 0.51 (0.17)[A,C]	+ 0.04 (0.19)[B]	+ 0.18 (0.15)
zLOOH+SOD	+ 0.10 (0.21)[D]	+ 0.20 (0.29)[C]	− 0.58 (0.33)[B,D]	+ 1.02 (0.26)[A,B,C]
zLOOH+SOD-CAT	+ 0.46 (0.25)	+ 0.26 (0.35)	− 0.40 (0.39)[D]	+ 1.06 (0.31)[C]
zLOOH+SOD+NOx	− 0.14 (0.26)[D]	+ 0.04 (0.36)[D]	− 0.85 (0.41)[D]	+ 1.41 (0.32)[A,B,C]
zLOOH+SOD+NOx+MDA	+ 0.10 (0.30)[C,D]	+ 0.14 (0.41)[D]	− 0.96 (0.46)[A,B,D]	+ 1.85 (0.37)[A,B,C]
zLOOH+SOD+NOx+AOPP	− 0.18 (0.30)[D]	+ 0.55 (0.40)[C]	− 0.81 (0.46)[B,D]	+ 1.59 (0.37)[A,C]

zLOOH+SOD computed as z transformation of LOOH (zLOOH) + zSOD

zLOOH+SOD-CAT computed as zLOOH + zSOD − zCAT

zLOOH+SOD+NOx computed as zLOOH + zSOD + zNOx

zLOOH+SOD+NOx+MDA computed as zLOOH + zSOD + zNOx + zMDA

zLOOH+SOD+NOx+AOPP computed as zLOOH + zSOD + zNOx + zAOPP

SOD superoxide dismutase, *LOOH* lipid hydroperoxides, *CAT* catalase, *NOx* nitric oxide metabolites, *MDA* malondialdehyde, *AOPP* advanced oxidation protein products

composite scores, we entered the latter in regression #2, Table 2. There was no significant effect of ASSIST hypnotics ($F = 1.06$, $df = 5/113$, $p = 0.386$), while ASSIST alcohol resulted in a significant effect ($F = 2.83$, $df = 5/113$, $p = 0.019$) although none of the univariate effects was significant. There were no significant effects of the drug state of the patients on the five composite scores, namely antidepressants ($F = 0.90$, $df = 5/107$, $p = 0.483$), lithium ($F = 0.70$, $df = 5/106$, $p = 0.628$), mood stabilizers ($F = 0.79$, $df = 5/106$, $p = 0.556$), and atypical antipsychotics ($F = 1.00$, $df = 5/107$, $p = 0.421$).

Best Predictions of O&NS Biomarkers

In order to delineate the best predictors of the biomarkers, we have carried out multiple regression analyses with the
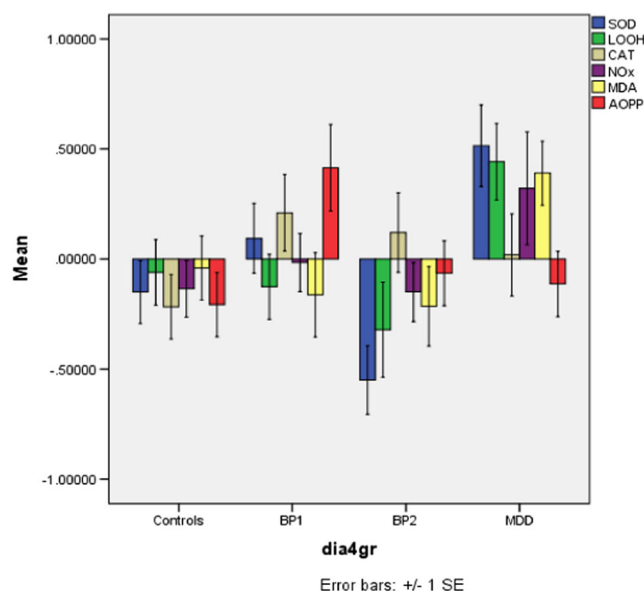

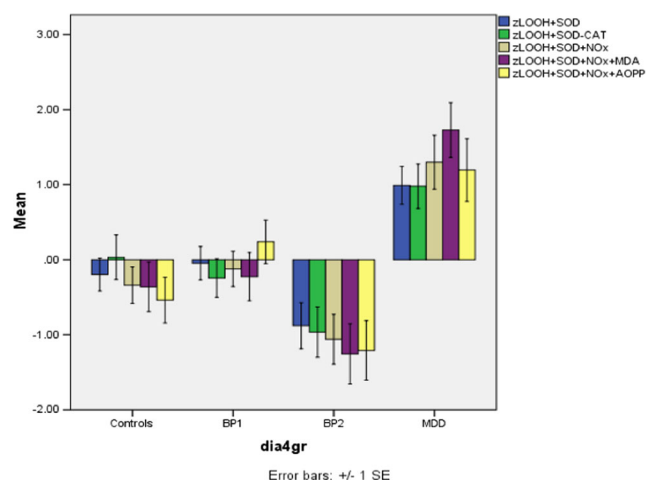**Fig. 2** The z-transformed values of the O&NS biomarkers in the four study groups

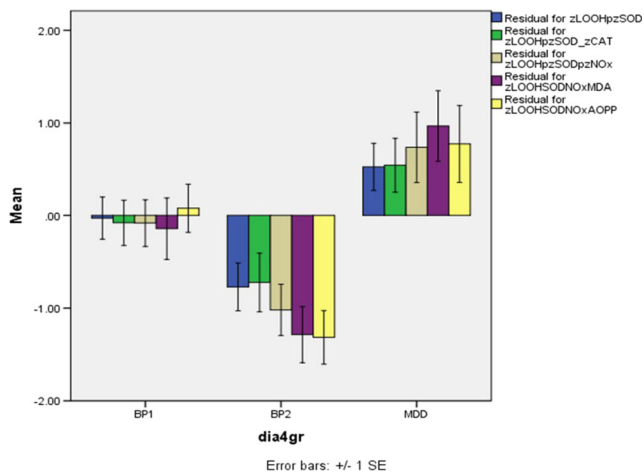**Fig. 3** The z transformed values of the five composite scores in the four study groups

Fig. 4 The residualized composite scores in the three mood disorder subgroups after adjusting for age, sex, body mass index, education, nicotine dependence, and the dichotomized HAM-D score (cut-off value 7)

biomarkers as dependent variables and the diagnostic groups (entered as four dummy variables: namely controls versus mood disorders, BPI versus the rest, BPII versus the rest, and depression versus the rest), HAM-D score, nicotine dependence (entered as three dummy variables, namely dependence versus no-dependence, mild dependence that is Fagerstrom score between 2 and < 6, and severe dependence, that is Fagerstrom score ≥ 6), age, sex, education, and BMI as explanatory variables. Table 4 shows that 21.4% of the variance in SOD1 was predicted by BPII, education (inversely associated), and age (positively). Of the variance in LOOH levels, 26.1% was explained by the HAM-D score, mild and severe nicotine dependence (positively), BPI and BPII (both negatively), and male sex. NOx was associated with major depression and nicotine dependence (positively). Of the variance in MDA, 15.2% was explained by the regression on major depression and years of education, while 22.6% of the
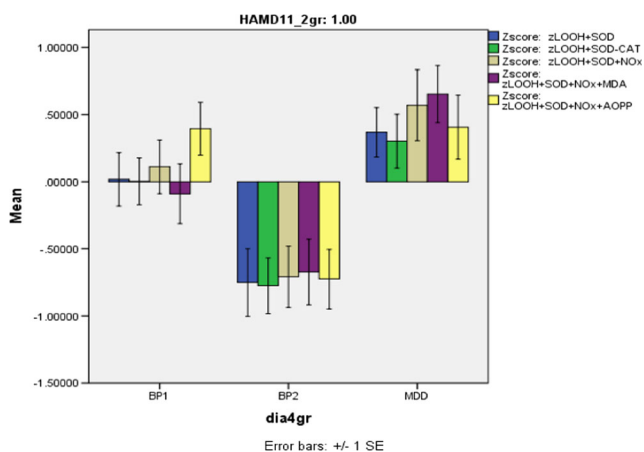
variance in AOPP levels was explained by HAM-D score, male sex, and BMI (all positively associated).

Of the variance in zLOOH+SOD, 29.8% was explained by major depression (positively), BPII (inversely), male sex, and years of education (inversely). Of the variance in zLOOH+SOD-CAT, 30.4% was explained by the regression on BPII (inversely), mild and severe nicotine dependence (positively), male sex, and education (inversely). Of the variance in zLOOH+SOD+NOx, 23.4% was explained by the regression on depression (positively), BPII, and education (both inversely). zLOOH+SOD+NOx+MDA was best predicted by major depression (positively), male sex, education, and BPII (both inversely). Of the variance in zLOOH+SOD+NOx+AOPP, 28.6% was explained by the regression on depression, HAM-D, BMI (all three positively), male sex, and BPII (inversely). The number of depressive and manic episodes was not significant in these regressions. There were also no significant univariate correlations between any of the biomarkers or composite scores and number of depressive and manic episodes.

Table 5 shows the outcome of a multiple regression analysis with the HAM-D as dependent variable and all 11 biomarkers together with age, sex, education, and number of episodes as explanatory variables. We found that 32.2% of the variance in HAM-D was explained by AOPP, number of depressive episodes, female sex, and education.

Discussion

The first major finding of this study is that patients with major depression in (partial) remission show significant aberrations in O&NS biomarkers as compared with HCs. These findings extend the results of recent meta-analyses indicating that depression is accompanied by indices of O&NS stress, including increased MDA or TBARS [35, 36]. Nevertheless, these meta-analyses reported that treatment with antidepressants significantly suppresses MDA/TBARS levels, while in our study, lipid peroxidation was also elevated in patients with depression who were in (partial) remission (HAM-D scores < 11). In addition, our study found higher SOD1 levels but no significant changes in catalase activity in participants with depression relative to HCs. The meta-analysis conducted by Liu et al. [35] reported a trend toward increased SOD levels in depression and no difference in catalase activity between patients with depression and controls. SOD catalyzes the partition of superoxide radicals into oxygen and hydrogen peroxides, which are further degraded into H_2O by catalase. SOD is a major protective antioxidant enzyme against the damaging effects of increased superoxide levels and additionally protects against peroxynitrite formation [66]. Nevertheless, SOD activity is accompanied by increased formation of hydrogen peroxides, which can generate other ROS, including

Fig. 5 z transformations of the five composite scores in patients with a HAM-D score < 11

Table 4 Results of multivariate regression analyses with oxidative and nitrosative stress biomarkers as dependent variables and diagnosis, nicotine dependence (ND), age, sex, body mass index, Hamilton Depression Rating Scale (HAM-A), and years of education as additional explanatory variables

Dependent variables	Explanatory variables	t	p	R (model)	F	df	p
SOD1	BPII	−2.97	0.004	0.214	10.69	3/118	< 0.001
	Age	+2.07	0.041				
	Education	−3.60	< 0.001				
LOOH	BPI	−2.41	0.018	0.261	6.79	6/115	< 0.001
	BPII	−2.49	0.014				
	Mild-ND	+2.86	0.005				
	Severe-ND	+2.29	0.030				
	HAM-D	+3.11	0.002				
	Sex	+2.92	0.004				
NOx	MDD	+2.73	0.007	0.070	4.75	2/120	0.013
	ND	+2.20	0.030				
MDA	MDD	+2.17	0.032	0.152	11.48	2/128	< 0.001
	Education	−3.88	< 0.001				
AOPP	HAM-D	+3.70	< 0.001	0.226	11.59	3/119	< 0.001
	Sex	+4.44	< 0.001				
	BMI	+2.17	0.032				
LOOH+SOD	MDD	+3.45	0.001	0.298	12.31	4/116	< 0.001
	BPII	−2.70	0.008				
	Sex	+2.23	0.028				
	Education	−3.83	< 0.001				
LOOH+SOD-CAT	BPII	−2.47	0.015	0.304	9.98	5/114	< 0.001
	Mild-NC	+2.51	0.014				
	Severe-NC	+2.80	0.006				
	Sex	+3.10	0.002				
	Education	−2.88	0.005				
LOOH+SOD+NOx	MDD	+3.91	< 0.001	0.234	11.92	3/117	< 0.001
	BPII	−2.26	0.026				
	Education	−2.32	0.022				
LOOH+SOD+NOx+MDA	MDD	+4.48	< 0.001	0.357	16.08	4/116	< 0.001
	BPII	−2.65	0.009				
	Sex	+2.38	0.027				
	Education	−4.17	< 0.001				
LOOH+SOD+NOx+AOPP	MDD	+3.65	< 0.001	0.286	9.23	5/115	< 0.001
	BPII	−2.86	0.005				
	HAM-D	+2.50	0.014				
	Sex	+3.69	< 0.001				
	BMI	+2.21	0.029				

zLOOH+SOD computed as z transformation of LOOH (zLOOH) + zSOD

zLOOH+SOD-CAT computed as zLOOH + zSOD − zCAT

zLOOH+SOD+NOx computed as zLOOH + zSOD + zNOx

zLOOH+SOD+NOx+MDA computed as zLOOH + zSOD + zNOx + zMDA

zLOOH+SOD+NOx+AOPP computed as zLOOH + zSOD + zNOx + zAOPP

Diagnosis is entered as four dummy variables, namely controls versus mood disorders, bipolar I (BPI) versus the rest, BPII versus the rest, and depression (MDD) versus the rest. Nicotine dependence is entered as three dummy variables, namely dependence versus no-dependence, mild dependence (that is Fagerstrom score between 2 and < 6) versus the rest, and severe dependence (that is Fagerstrom score ≥ 6) versus the rest

SOD superoxide dismutase, *LOOH* lipid hydroperoxides, *CAT* catalase, *NOx* nitric oxide metabolites, *MDA* malondialdehyde, *AOPP* advanced oxidation protein products

the very reactive hydroxyl or metal-associated radicals [66, 67]. Moreover, oxidative stress and inflammatory triggers (e.g., T cell activation) may enhance SOD activity as well as its de novo synthesis especially in early stages of an injury [68, 69]. This explains why enhanced SOD activity may constitute a compensatory mechanism protecting against overwhelming amounts of superoxide.

In order to decipher the association between mood disorders and nitro-oxidative pathways, we computed specific composite scores that may reflect ROS and LOOH formation (zLOOH+SOD) and the protective effects of catalase on ROS/LOOH production (zLOOH+SOD-CAT). The increased levels of zLOOH+SOD and zLOOH+SOD-CAT found in depressed patients indicate an increased production of ROS/

Table 5 Results of multivariate regression analyses with the Hamilton Depression Rating Scale (HAM-D) score as dependent variable and number of depressive episodes, oxidative and nitrosative stress biomarkers, nicotine dependence, age, sex, body mass index, and years of education as explanatory variables

Dependent variables	Explanatory variables	t	p	R (model)	F	df	p
HAM-D	No. of depression episodes	+ 5.16	< 0.001	0.322	14.36	4/121	< 0.001
	AOPP	+ 3.04	0.003				
	Male sex	− 2.43	0.017				
	Education	− 2.13	0.035				

AOPP advanced oxidation protein products

LOOH, which appears to be insufficiently neutralized by catalase. These findings are in agreement with the meta-analysis by Liu et al. [35], which reported higher peroxide levels and no significant changes in catalase levels in individuals with depression compared to HCs.

This study detected a significant (albeit weak) association between depression and increased NOx levels. Although an increased NO production was not observed in the meta-analysis of Liu et al. [35], some other studies reported increased NOx in depression. Gomes et al. [40] found that depression co-occurring with chronic apical periodontitis is accompanied by increased NO metabolites which are significantly correlated with depression severity. Furthermore, in pregnant women, increased NOx levels are significantly associated with IgM responses to NO-adducts, indicating amplified nitrosylation [70], while NOx as well as IgM responses directed to NO-adducts are significantly associated with a history of mood disorders. Several levels of evidence point to a role of NO in the pathophysiology of depression [2, 3, 71]. Nitrosylation with increased S-nitrosothiol (SNO) levels regulate neuroimmune systems and when moderate may protect neuronal systems, whilst hypernitrosylation may contribute to neuroprogressive and neurodegenerative processes [44, 72].

We also constructed a new composite score reflecting increased ROS/RNS production (namely zLOOH+SOD+NOx). The latter score was increased in depression (even after adjusting for HAM-D scores) indicating increased nitro-oxidative stress and a higher potential to generate hydroxyl radicals and peroxynitrite, which have caused damage to lipid membranes [64]. These findings may explain previous evidence that depression is accompanied by signs of nitro-oxidative damage to fatty acid membranes [18–20, 22, 24, 25] as well as higher protein oxidation and nitration [2, 3].

We also found that major depression is characterized by increased composite scores reflecting activation of the pathway from formation of ROS and lipid peroxidation to reactive aldehydes with long-lasting detrimental consequences (zLOOH+SOD+NOx+MDA) and protein nitro-oxidation (zLOOH+SOD+NOx+AOPP). These findings confirm that depression is accompanied by increased nitro-oxidative stress

that may drive damage to proteins and lipids. In addition, our results indicate that that over-activation of these pathways may be evident even during (partial) remission. Previous research detected that TBARS is also increased in the euthymic phase of depression and, therefore, TBARS was described as a trait biomarker of depression rather than a state marker [8]. Our findings are also in agreement with the knowledge that depression is characterized by lowered antioxidant levels which (a) prevent and protect against lipid peroxidation, including lowered LCAT activity, HDL-cholesterol, vitamin E, as well as coenzyme Q10 and paraoxonase 1 activities [9–12, 16, 17, 35], and (b) remove and repair lipid hydroperoxide lesions, including glutathione peroxidase, HDL cholesterol, and LCAT [3, 9, 10, 35, 63].

The second major finding of this study is that there are significant differences in O&NS pathways between patients with BPI and depression as well as HCs. Recent meta-analyses show that BD is accompanied by signs of lipid peroxidation, protein oxidation (as indicated by elevated protein carbonyl levels), NO production, and increased nitrotyrosine levels, indicating enhanced nitration [37, 38, 73]. A previous study reported higher SOD activity in euthymic BD patients [45], while we established increased SOD1 activity in MDD patients only. The results of the current study show that protein oxidation (AOPP levels) is significantly increased in patients with BPI and depression as compared with HCs, but that the nitro-oxidative pathway from ROS+RNS to MDA formation is less activated in BPI than in MDD. Previously, it was shown that increased TBARS in BD is a biomarker of acute depressive and manic episodes and also a possible stage biomarker of BD [8, 74]. Thus, the inclusion of patients in (partial) remission (this study) may explain the lower MDA levels in BPI herein observed. Increased protein oxidation was previously observed in BD as measured with protein carbonyls, expressed as C=O contents [38]. Nevertheless, protein carbonylation (C=O) and formation of AOPP (O=C) are different pathways. Protein carbonyls are generated from direct protein carbonylation via the effects of hydrogen peroxides, while an indirect mechanism involves non-oxidative reactions with oxidized lipids that contain carbonyl radicals and

cleavage of protein backbones as well as α-amidation [75–77]. AOPPs, on the other hand, are generated via increased production of ROS and peroxynitrite coupled with increased myeloperoxidase activity during chlorine stress [42, 76]. The results of the current study suggest that increased protein oxidation with generation of AOPPs is associated with BPI and MDD even in (partial) remission, while illness severity seemed to be accompanied by higher AOPP levels.

The third major finding of this study is that patients with BPII show significantly lower SOD1 and LOOH levels and lower zLOOH+SOD+NOx, zLOOH+SOD+NOx+MDA, and zLOOH+SOD+NOX+AOPP scores when compared to all other groups. In addition, BPII patients exhibited lower AOPP, zLOOH+SOD, zLOOH+SOD+NOx+MDA, and zLOOH+SOD+NOX+AOPP scores as compared to BPI patients and a significantly lower zLOOH+SOD+NOx+MDA score than HCs. These marked differences among patients with BPI and BPII in most O&NS pathways measured herein have not been previously reported. A previous study found that TBARS levels to be significantly increased in BPI and BPII patients compared to controls, while no significant differences in TBARS between both BD subtypes were observed [8]. These discrepant findings may be explained by differences in study samples regarding phase of illness (acute versus partial remission). Our results show that the O&NS pathway from ROS production to nitro-oxidative damage is attenuated in (partially) remitted BPII patients. One hypothesis is that patients with BPII could be protected against nitro-oxidative stress or display more adequate removal and repair mechanisms of lipid peroxide lesions and that this could explain its milder clinical phenotype as compared to BPI. Possible protective and repair mechanisms could be enhanced antioxidant defenses and better regulation through LCAT or IgM-mediated autoimmune responses [70]. However, no evidence is available to support this tentative hypothesis. It should be also noted that our findings are consistent with other levels of evidence that point to biological differences between BPI and BPII although this remains a relatively unexplored field [78, 79].

Another finding is that O&NS pathways in mood disorders could be modulated by effects of sex, education, and nicotine dependence. Thus, peroxides and AOPP levels and all composite scores were significantly higher in males than females. Previously, it was observed that plasma peroxides, but not IgG/IgM responses to oxidized LDL, are significantly greater in males than females [23] and that males show greater responses in ROS production than females [80]. The current study found that male sex is specifically associated with increased lipid hydroperoxide production, while there are no significant sex-linked differences in SOD1 or catalase activities. We observed that education is inversely associated with SOD1, the zLOOH+SOD composite score, and MDA formation, suggesting that education has a protective effect on the

generation of ROS, thereby protecting against lipid peroxidation and reactive aldehyde production. Such effects may likely be explained by education resulting in a healthier lifestyle, which may increase protection or repair mechanisms through for example nutrition and exercise [81, 82]. Previous studies reported a strong impact of smoking and nicotine dependence on different O&NS pathways [83, 84] and that the increased risk for development of mood disorders in smokers is in part associated with smoking-induced oxidative stress pathways [85]. Nevertheless, in the current study, there were only mild associations between nicotine dependence (mild or severe) and increased LOOH levels, indicating that current smoking may somewhat increase lipid peroxidation without significant effects on reactive aldehyde formation and oxidative damage to proteins. Although age is significantly associated with increased levels of some, but not all, O&NS pathways and lowered antioxidant enzyme defenses [86, 87], the current study was unable to find relevant associations among age and the pathways measured herein. One explanation is that a stronger impact of diagnosis, sex, and education on O&NS pathways could have blurred the effects of age.

The results of the current study should be interpreted within its limitations. Firstly, this is a cross-sectional study, and therefore, no firm causal inferences can be established. Second, we assayed peripheral biomarkers and the extent to which these findings reflect brain alterations remain imprecise.

The current study showed that there is another fundamental problem with classifications of mood disorders, which often lump both BP types together, while in fact, our results show that both BPI and BPII may be quite different biological entities, which in addition differ from major depression. Current psychiatric nosological diagnoses are heavily debated [88] because mental disorders as defined by the DSM-IV-TR and DSM-5 lack statistical and biological validation [89–91]. Most diagnostic categories based on syndromal phenomenology will soon become a historical footnote [92, 93]. The delineation of trans-diagnostic phenotypes as defined in the NIMH Research Domain Criteria (RDoC) system may provide a somewhat better outcome [88, 94]. More specifically, physiosomatic symptoms have emerged as a new phenotype and the identification of this construct across mood disorders as well as somatizing and psychotic disorders has aided in the identification of more precise diagnostic biomarkers, including activated nitro-oxidative pathways [91, 95, 96]. Nevertheless, both consensus-based DSM classifications and the RDoC system miss our point that (a) classifications of psychiatric phenomenology should be derived from pattern recognition methods including supervised and unsupervised learning, which should be used to refute or consolidate existing classifications and detect new classifications, which additionally should be externally validated by biomarkers [91, 97], and (b) trans-diagnostic phenotypes should be derived by pattern recognition methods including deep learning to

discover pathophysiologically delineated endophenotypes [96, 98]. Nevertheless, the sample size of the current study did not allow the conduction of unsupervised machine learning analyses in order to provide a more accurate indication of the possible clinical utility of the biomarkers herein measured as a means to aid in the differentiation of mood disorders or to detect relevant endophenotypes.

In conclusion, the current study indicates that alterations in specific nitro-oxidative pathways may differ among depression, BPI, and BPII. If replicated, these findings open relevant perspectives including the development of a panel of biomarkers that could aid in the differentiation of mood disorders. Furthermore, future studies should explore possible differences in mood disorders in other biomarkers related to O&NS including but not limited to myeloperoxidase, iNOS activity, protein carbonyls, and IgM-mediated autoimmune responses to oxidatively formed neoepitopes.

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