

Increased levels of Serum Nitrogen oxides are indicators of Post-treatment response in Mood disorder patients with Acute episodes

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ABSTRACT

Background:

Nitrogen Oxides (NOx) has an important role in mood disorders. This study aims to investigate whether serum NOx levels are associated with bipolar disorder, manic episodes (BD-ME) and major depressive disorder, depressive episodes (MDD-DE), and to evaluate the impact of treatments on NOx levels and symptoms severity in BD-ME and MDD-DE patients.

Methods:

We enrolled 20 patients with BD-ME, 18 with MDD-DE, and 80 healthy subjects. Serum NOx levels were measured with assay kits. Symptom severity was evaluated using the Young Mania Rating Scale (YMRS) for BD-ME and the Hamilton Depression Rating Scale (HAM-D) for MDD-DE. We collected and analyzed patients' NOx, YMRS, and HAM-D data while pre-treatment (admission) and post-treatment (discharge).

Results:

Serum NOx levels of the healthy controls were higher than patients with BD-ME and lower than patients with MDD-DE, but the difference was not statistically significant. Serum NOx levels were both increased in BD-ME ($p=0.0047$) and in MDD-DE ($p=0.0398$) patients after treatment. The changes of NOx levels were not associated with the changes of YMRS scores in BD-ME patients after treatment. However, the changes of NOx levels were negatively correlated with the changes of HAM-D scores in MDD patients after treatment ($p=0.0473$).

Conclusions:

Our results suggest that increased levels of serum NOx are indicators in patients with BD-ME and MDD-DE after treatment. In addition, the changes of serum NOx levels might be measuring biomarkers for assessment of treatment response in MDD-DE patients.

Keywords

Nitrogen oxides, BD, Bipolar disorder, Mania, MDD, Depressive disorder, Depression

Introduction

Mood disorder, a category of severe psychiatric illness affecting many in the general population, mainly includes bipolar disorder (BD) and

major depressive disorder (MDD). A number of assessment tools are used to diagnose or evaluate the severity of bipolar disorder with manic episode (BD-ME), bipolar disorder

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with depressive episode (BD-DE), and major depressive disorder with depressive episode (MDD-DE) [1-3]. However, these psychiatric rating scales should be used by psychiatrists or well-trained clinical professionals. Clinicians lack biological indicators which might stand for diagnosis and prognosis after treatment [4].

Nitrogen Oxides (NO_x) has been considered as a potential biomarker for diagnosis assisting. It is an important neurotransmitter involved in the pathophysiology of many neuropsychiatric disorders, such as BD, MDD, schizophrenia, and drug addiction [5-8]. Several studies have used NO_x levels in the blood as biomarkers to detect BD-ME [9,10], BD with a euthymic state SS[11], BD-DE [12], and MDD-DE [13-15]. However, the results have contradictory outcome. Furthermore, few studies have investigated the changes of NO_x levels after treatment in BD-ME and MDD-DE patients, and showed unsatisfactory results [16-18].

The aim of our study is to verify the hypothesis, the serum NO_x levels as diagnosis tool in BD-ME and MDD-DE patients compared with healthy controls. Moreover, we investigate the serum NO_x levels and evaluate the severity of BD-ME by The Young Mania Rating Scale (YMRS)¹ and MDD-DE by the 17-item Hamilton Depression Rating Scale (HAM-D) [19] in pre-treatment and post-treatment state. Then we assess the relationship between the changes of serum NO_x levels and symptom severity scores after treatment in BD-ME and MDD-DE patients.

Materials and Methods

■ Patients

All BD-ME and MDD-DE patients in the psychiatric inpatient ward of Kaohsiung Chang Gung Memorial Hospital were enrolled. They met the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria for BD-ME and MDD-DE, and were diagnosed by psychiatrists. Background data including gender, age, weight, height, and body mass index (BMI) were recorded. Serum NO_x levels at pre-treatment (admission) and post-treatment (discharge), and during hospitalization were collected. The severity of manic symptoms in BD-ME and depressive symptoms in MDD-DE were evaluated using the YMRS¹ and the 17-item HAM-D [2,19], respectively. Severity was assessed at pre-treatment and post-treatment. All patients were aged from 18 to 65, and

underwent blood pressure, routine blood tests, electrocardiogram (EKG), and chest X-ray at admission. Those with currently **unstable** physical problems, a history of heavy smoking, or a history of substance abuse were excluded. Twenty-three BD-ME patients and 21 MDD-DE patients initially were enrolled. They were treated with antipsychotic agent, mood stabilizer, and antidepressant according to their symptoms. The dosage of medications was titrated to treatment dose within 2 weeks, and then maintained to date of discharge. Three BD-ME patients and 3 MDD-DE patients dropped out of the study; the remaining 20 BD-ME patients and 18 MDD-DE patients were included.

The healthy controls were recruited at Kaohsiung Chang Gung Memorial Hospital. Similar background data were collected. Those with a history of mental disorders or severe medical disease were excluded. All the controls were free of medication. Eighty individuals finally were included in this study.

The study was carried out at Kaohsiung Chang Gung Memorial Hospital from November 2012 to October 2014, and was approved by the Institutional Review Board of the hospital and performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All subjects signed informed consent forms after receiving a full explanation of the study.

■ Laboratory data

Venous blood samples were collected in the morning after overnight fasting (at least 8 hours). The serum samples were separated by centrifugation at 3000 g for 10 min, and then stored at -80°C for further analysis soon after blood pumping. A nitrate/nitrite colorimetric assay kit was used to assay serum NO_x (Cayman Chemical Company, Ann Arbor, USA). All analyses were performed at the same laboratory and according to the manufacturer's protocol. The website of the protocol was <https://www.caymanchem.com/product/780001>.

■ Statistical analysis

Continuous variables were expressed as mean ± standard deviation. The Kolmogorov-Smirnov test was used for normality. The independent samples t-test was used to compare patient and control data that were normally distributed, and the Mann-Whitney U test was used for data that were not normally distributed. Analysis of covariance (ANCOVA) was used to compare the serum NO_x levels at pre-treatment between

patients and controls while controlling for the gender, age, and BMI. Paired samples t-test was used to analyze the changes of serum NOx levels, YMRS scores and HAM-D scores in the patients. Pearson’s correlation coefficient was used to evaluate the relationship between serum NOx levels and YRMS scores or HAM-D scores. A *p* value of less than 0.05 was used to indicate statistical significance. All data were analyzed using MedCalc Statistical Software version 16.2.0 (MedCalc Software bvba, Ostend, Belgium; <https://www.medcalc.org>; 2016).

Results

Table 1 shows the characteristics of patients and controls. A total of 118 subjects met the recruitment criteria, including 20 BD-ME patients (12 males and 8 females), 18 MDD-DE patients (3 males and 15 females), and 80 controls (30 males and 50 females). There was a significant age difference between the BD-ME patients *vs* controls (*p*=0.0025) and the MDD-DE patients *vs* controls (*p*<0.0001). There were no significant differences in the other background data (weight, height, and BMI) between BD-ME patients *vs* controls and MDD-DE patients *vs* controls (*p*>0.05). The mean hospitalization durations of the patients in the BD-ME and MDD-DE groups were 25.8±5.0 days and 24.8±6.0 days, respectively. Serum NOx levels were reduced in BD-ME patients *vs* control (25.8±18.6 vs. 21.9±12.8) and increased in MDD-DE patients *vs.* controls (34.6±21.0 vs. 25.8±18.6), but without significant differences (*p*=0.7110 and *p*=0.0766, respectively). ANCOVA showed that the age had no significant effect on serum NOx levels between BD-ME patients *vs* controls (*F*=1.441, *p*=0.233) and MDD-DE patients *vs* controls (*F*=2.058, *p*=0.155) after controlling the gender, age, and BMI (Data not shown). **Table 2** shows the medications and maintenance dose received by the BD-ME and MDD-DE patients.

The BD-ME patients showed significantly increased levels of serum NOx at post-treatment compared with pre-treatment and the healthy controls (*p*=0.0047 and *p*=0.0224). The BD-ME patients also had significant improvement in YMRS scores between pre-treatment and post-treatment (*p*<0.0001). The MDD-DE patients presented significantly increased levels of serum NOx at post-treatment compared with pre-treatment and the healthy controls (*p*=0.0398 and *p*=0.0009). The MDD-DE patients also had significantly improved HAM-D scores from pre-

Table 1: Characteristics of BD-ME patients, MDD-DE patients, and healthy controls in the study.

	BD-ME (n=20)	MDD-DE (n=18)	Controls (n=80)
Gender (Male/Female)	12/8	3/15	30/50
Age (years)	40.8 ± 12.3 ^a	50.9 ± 6.7 ^b	31.7 ± 5.5
Weight (kg)	61.5 ± 8.7	63.3 ± 16.8	59.9 ± 11.8
Height (cm)	165.1 ± 7.1	160.6 ± 6.7	163.9 ± 8.4
BMI (kg/m ²)	22.6 ± 3.3	24.6 ± 6.5	22.2 ± 3.0
NOx ^c (μmol/L)	21.9 ± 12.8	34.6 ± 21.0	25.8 ± 18.6
Hospitalization (days)	25.8 ± 5.0	24.8 ± 6.0	NA

Data values are given as mean ± standard deviation
^a*p*<0.01, BD-ME *vs* Controls; ^b*p*<0.0001, MDD-DE *vs* Controls; ^cMean serum nitrogen oxides level in pre-treatment or healthy controls

Abbreviation: BD-ME = bipolar disorder with manic episode; MDD-DE = major depressive disorder with depressive episode; BMI = body mass index; NOx = nitrogen oxides; NA = not applicable

treatment to post-treatment (*p*<0.0001) (**Table 3**) (**Figure 1A &1B**).

Serum NOx levels and YMRS scores of pre-treatment BD-ME patients showed no significant correlation (*r*=0.1391, *p*=0.5588). Also, no significant correlation was noted between serum NOx levels and HAM-D scores in pre-treatment MDD-DE patients (*r*=-0.0045, *p*=0.9858). (Data not shown)

The changes of serum NOx levels and YMRS scores in BD-ME patients after treatment showed no significant correlation (*r*=-0.1831, *p*=0.4396) (**Figure 2A**). However, a significant correlation was noted between changes of serum NOx levels and HAM-D scores in MDD-DE patients after treatment (*r*=-0.4732, *p*=0.0473) (**Figure 2B**).

Discussion

Our study found that serum NOx levels were non-significantly reduced and increased in BD-ME and MDD-DE patients, respectively, while treatment significantly increased these values in both groups. We also found that the changes of NOx levels in MDD-DE patients after initial treatment had a negative correlation with the changes of HAM-D scores.

■ Blood NOx levels in BD, MDD, and healthy subjects

Few clinical trials in past decades have investigated blood NOx levels in BD-ME patients. Savas *et al.* found that the mean plasma NOx level in BD-ME patients (*n*=44) was significantly higher than that in controls [9]. Case-control studies by Yanik *et al.* (*n*=43 in the BD-ME group and *n*=31 in the control group) and Gergerlioglu *et al.* (*n*=29 in the BD-ME group and *n*=30 in the control group) had similar results [16,20].

Table 2: Medications used by the BD-ME patients and MDD-DE patients in the study.

BD-ME (n=20)		MDD-DE (n=18)	
Patient	Medications	Patient	Medications
1	Haloperidol 5 mg + Lithium 900 mg	1	Fluoxetine 20 mg
2	Olanzapine 20 mg + Lithium 900 mg	2	Agomelatine 50 mg + Quetiapine 25 mg
3	Risperidone 3 mg + Lithium 1200 mg	3	Duloxetine 60 mg + Quetiapine 150 mg
4	Risperidone 4 mg + Valproate 1000 mg	4	Bupropion 150 mg + Fluoxetine 40 mg
5	Haloperidol 5 mg + Valproate 1200 mg	5	Fluoxetine 80 mg + Quetiapine 500 mg
6	Risperidone 3 mg + Lithium 900 mg	6	Fluoxetine 60 mg + Aripiprazole 2.5 mg
7	Valproate 1000 mg	7	Paroxetine 40 mg + Aripiprazole 5 mg
8	Risperidone 3 mg + Lithium 1200 mg	8	Agomelatine 25 mg
9	Olanzapine 20 mg + Valproate 1000 mg	9	Escitalopram 20 mg
10	Risperidone 3 mg + Lithium 1200 mg	10	Duloxetine 30 mg + Quetiapine 100 mg
11	Aripiprazole 15 mg + Valproate 400 mg	11	Escitalopram 10 mg + Trazodone 50 mg
12	Clozapine 50 mg + Valproate 1000 mg	12	Paroxetine 40 mg
13	Paliperidone 3 mg + Valproate 1200 mg	13	Venlafaxine 225 mg
14	Risperidone 6 mg + Valproate 1200 mg	14	Agomelatine 50 mg
15	Aripiprazole 15 mg + Lithium 600 mg	15	Fluoxetine 40 mg
16	Risperidone 3 mg	16	Escitalopram 15 mg + Amitriptyline 50 mg
17	Valproate 1000 mg	17	Mirtazapine 60 mg + Quetiapine 50 mg
18	Risperidone 4 mg + Lithium 600 mg	18	Bupropion 300 mg
19	Risperidone long-acting injection 25 mg + Lithium 600 mg		
20	Risperidone long-acting injection 37.5 mg + Lithium 600 mg		

Table 3: Mean changes in NOx levels, and YMRS and HAM-D scores of BD-ME and MDD-DE patients after treatment.

	BD-ME (n=20)		MDD-DE (n=18)	
	NOx (µmol/L)	YMRS	NOx (µmol/L)	HAM-D
Pre-treatment	21.9 ± 12.8	35.3 ± 7.3	34.6 ± 21.0	27.4 ± 5.9
Post-treatment	38.8 ± 24.1	3.2 ± 2.9	51.8 ± 36.4	8.3 ± 5.7
p value	0.0047**	<0.0001**	0.0398*	<0.0001**

Data values are given as mean ± standard deviation

*p<0.05; **p<0.01

Abbreviation: NOx = nitrogen oxides; YMRS = Young Mania Rating Scale; HAM-D = Hamilton Depression Rating Scale; BD-ME = bipolar disorder with manic episode; MDD-DE = major depressive disorder with depressive episode

However, a study by Ozcan *et al.* found decreased plasma NOx levels in the pre-treatment bipolar patient group compared to the control group [10]. Differing from previous findings, our study showed no significant differences between BD-ME patients and controls. The reasons for the discrepancy might be differences in race, in sample sizes, and in severity of BD-ME.

The role of blood NOx levels in MDD-DE has been debated since the 2000s. Some studies proposed that blood NOx levels had a positive association [13,17,21-27], others suggested no significant association [18], and still others reported a negative association with MDD-DE [14,15,28,29]. Our study found higher mean serum NOx levels in MDD-DE patients than in healthy controls, but without a significant difference. These discrepancies might be the result of differences in case heterogeneity. For instance,

patients in some studies had comorbid physical disease (colorectal carcinoma or leukemia) [26,27] or other psychiatric disorders [14,18,28]; some had been diagnosed with a first episode of MDD [13,21,29] or had recurrent depressive episodes of MDD25, or had attempted suicide [22,23]; one study enrolled only female groups [15], and another only Han Chinese subjects [17]. Our study population was purely mood disorder without major physical problem, but we didn't distinguish first or recurrent episode, gender, and severity of MDD. All these factors might affect blood NOx levels [18,22,25,29,30].

Taken together, these studies and our research had inconsistent results of blood NOx levels in BD-ME and MDD-DE patients compared with healthy controls. Gene polymorphism may structurally interpret them. Nitric oxide (NO) is synthesized from L-arginine and oxygen (O₂)

with the help of nitric oxide synthases (NOSs) [7]. The neuronal isoform of NOS (encoded as NOS1) is the main source of NO in humans, and a certain single nucleotide polymorphisms (SNP) in the NOS1 might pathologically disturb NO production by its activity [31]. NOS1 has a complex haplotype structure and diverse characteristics, which might have led to the results of different NOx levels in BD-ME and MDD-DE patients [11,12,18,27,29].

■ **The changes of blood NOx levels in BD and MDD patients**

Gergerlioglu et al. evaluated the changes of serum NOx levels in acute-phase BD-ME patients during treatment. They found mildly higher NOx levels in BD-ME patients on the 30th day (57.05±15.47 µmol/L) than on the 1st day (54.87±6.03 µmol/L), but without significance¹⁶. Ozcan et al. also reported higher NOx levels in the erythrocytes of patients with affective disorders at post-treatment (298.0±28.9 nanomol/g) than at pre-treatment (267.6±19.1 nanomol/g) [10]. Our results showed that the serum NOx levels in BD-ME patients were significantly increased after treatment, and supported previous findings.

Besides, two studies from Turkey investigated the relationship between NOx levels and Bech-Rafaelson Mania Scale scores [32] in pre-treatment BD-ME patients, and reported no significant correlations [9,16]. Our data also supported these results, finding no significant correlation between serum NOx levels and YMRS scores in pre-treatment BD-ME patients. Furthermore, we found that the changes of serum NOx levels were not correlated to the improvement of YMRS scores in BD-ME patients after treatment. Based on the above results, increased levels of serum NOx might not relate with initial status or symptoms improvement in BD-ME patients, which could be explained by mood stabilizer using. Previous studies indicated that lithium and valproic acid would induce NOx synthase and elevated NOx levels after these medications use, and supported the results [33,34]. Almost all patients in our study took mood stabilizer during hospitalization, and then they were found increased the serum NOx levels after treatment, which were not correlated to the change of YMRS scores.

Few studies have compared the changes of NOx levels pre-treatment and post-treatment in MDD-DE patients [17,18]. Herken et al. (n=32) detected serum NOx levels at pre-treatment and

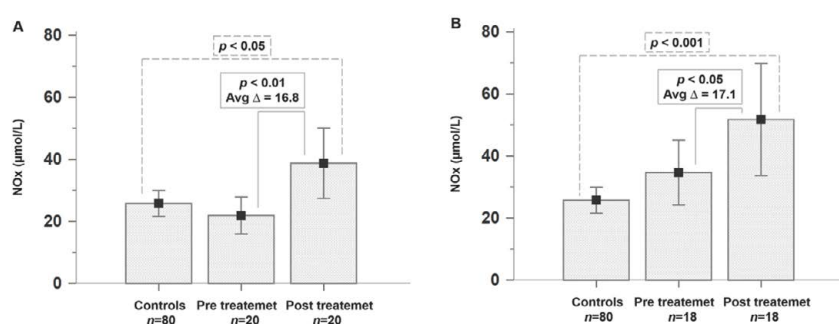


Figure 1: The mean serum nitrogen oxides (NOx) levels in healthy controls and patients during pre-treatment and post-treatment periods

(A): Healthy controls and bipolar disorder patients with manic episodes (BD-ME); (B): Healthy controls and major depressive disorder patients with depressive episodes (MDD-DE)

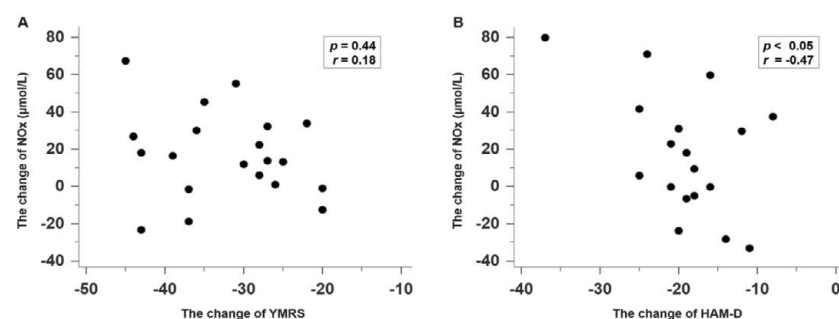


Figure 2: Changes in serum nitrogen oxides (NOx) levels and symptoms rating scores of patients after treatment.

(A): Bipolar disorder with manic episode (BD-ME) patients rated by the Young Mania Rating Scale (YMRS); (B): Major depressive disorder with depressive episode (MDD-DE) patients rated by the Hamilton Depression Rating Scale (HAM-D)

post-treatment, in a study period of 8 weeks, and found significantly decreased NOx levels [18]. Lu et al. revealed that NOx levels remained elevated, without a significant difference (n=7, p ≥ 0.128), even after 2 months of fluoxetine treatment [17]. Baranyi et al. indicated increased NO production might be an indicator of remission in major depression patients [35]. Our data revealed significantly increased serum NOx levels in MDD-DE patients after initial treatment. Two differences in study methodologies might account for above results. One, different antidepressants could produce the same anti-depressant effects but they might decrease or elevate NOx levels [36]. In our study, we used 8 kinds of antidepressant in [18] MDD-DE patients with obvious heterogeneity, so it might indicate the increasing trend of NOx levels after “general” antidepressant treatment. Second, the length of treatment time was different. We collected NOx data at around 4 weeks compared to the 8 weeks of previous studies. The difference

in time of NOx collection might have affected the results.

Hence, we used another new perspective for the trend of NOx levels after treatment. We investigated the relationship between the changes of NOx levels and the degree of HAM-D improvement after treatment, which was more valuable than duration after treatment, and our data found a significant negative association between them. The meaning of our result suggested the changes of NOx levels might be an objective marker for assessment improvement in MDD-DE patients after initial treatment.

One NOx protective hypothesis explained increased NOx level after treatment. NOx caused a reaction with superoxide (O₂⁻) to form highly oxidant and neurotoxic peroxynitrite (ONOO⁻) [37]. ONOO⁻ can attack neural cells and has a calamitous role in neuro-degeneration and mood switching [7,38]. In contrast, NOx had a benefit in functional recovery and synaptic plasticity from cerebral ischemia in some studies [39-41]. The patients showed increasing NO level after treatment, which regulated endothelial function via arginase activity, L-arginine/ asymmetric dimethylarginine (ADMA) ratio, and global arginine bioavailability [35]. Taken together, NOx was not toxic, and played a neuroprotective role via endothelial regulation system.

Limitations

Several limitations should be highlighted. First, measuring NOx levels in blood samples from different locations, such as brain tissue and peripheral blood (RBC, plasma, or serum), may result in some discrepancies. We collected NOx data of all subjects from peripheral venous serum. Second, we prescribed heterogeneous antipsychotic agents, mood stabilizers, and antidepressants for each patient. Not prescribing the same medication may have influenced the results. Serum NOx may be increased after antipsychotic treatment [42], increased while

mood stabilizers using [33,34], and increased or unaffected after using antidepressant [43,44]. Third, age differences may influence oxidative stress biomarkers and result in discrepancies [45]. There was a significant difference in mean age of the BD-ME and MDD-DE patients and healthy controls in our study, but multiple regressions showed that it was not a significant factor affecting NOx levels. Fourth, sample sizes were too small to group individuals by gender, BMI, and medications, which may yield differences in NOx levels and hinder adequate statistical analysis [46]. Future studies should include follow-up NOx data of patient different states, and use large sample sizes to distinguish different kinds of medications, gender, age, and BMI, to determine the association between mood disorder and serum NOx levels.

Conclusions

In this study, we found that the levels of serum NOx in both BD-ME and MDD-DE patients at post-treatment were significantly increased compared to pre-treatment. Increased levels of serum NOx were indicators of post-treatment in mood disorder patients with acute episodes. In addition, the changes of serum NOx levels in MDD-DE patients after treatment had a negative correlation to the changes of HAM-D scores. The changes of serum NOx levels might be the measuring biomarker of symptoms improvement in MDD-DE patients.

Declaration of interests

There is no conflict of interest regarding publication of this paper. We obtained no financial support from any drug company.

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