Alterations in Plasma Nitric Oxide Level and Arginase Activity During The Treatment of Bipolar Depressive Episode

Nurdan Özlü Ceylan[1], İclal Geyikli Çimenci[2], Ayşe Kılınçaslan[3], Feridun Bülbül[4], Haluk A. Savaş[5]

ÖZET:

İki uçlu bozuklukta depresif dönemin tedavisi sırasında plazma nitrik oksit düzeyi ve arginaz aktivitesindeki değişiklikler

Amaç: Nitrik oksitin (NO) iki uçlu bozukluğun (İUB) patofizyolojisinde rol oynadığı çeşitli çalışmalarda gösterilmiştir. Arginaz ve NO'i sentezleyen NO sentaz enzimleri ortak substrat olarak L-arginini kullanır. Bu nedenle arginaz enziminin aktivitesi NO'in düzenlenmesinde önemli olabilir. Yapılan çalışmalarda İUB'de NO düzeyleri artmış olarak bulunsa da, bu hastalardaki arginaz enzimi aktivitesi ile ilgili tablo halen belirsizdir. Bu çalışmada prospektif olarak iki uçlu bozukluk-depresif epizod (İUB-DE) hastalarında NO seviyeleri ile birlikte arginaz aktivitesinin değerlendirilmesi amaçlanmıştır.

Yöntem: DSM-IV'e göre tanı konulan 33 İUB-DE hastası ve bu hastalarla yaş, cinsiyet ve sigara kullanımı yönünden eşleştirilmiş sağlıklı gönüllü kontroller çalışmaya dahil edildi. Hasta grubunda herhangi bir tedavi başlamadan önce serum NO düzeyleri ve arginaz aktiviteleri ölçüldü, bulunan değerler kontrol grubunun sonuçlarıyla karşılaştırıldı. Hastalara doğal döneme özgü tedaviler (farmakoterapi ve gereğinde elektrokonvülzif terapi) uygulandı. NO düzeyleri ve arginaz aktiviteleri tedavinin 30. gününde tekrar değerlendirildi. Klinik sonlanım Hamilton Depresyon Ölçeği ile değerlendirildi.

Bulgular: Hastaların tedavi öncesi NO düzeyi ve arginaz aktivitesi sağlıklı gönüllü kontrollerden istatistiksel olarak anlamlı bir biçimde daha yüksekti (ikisi için de $p<0.001$). 30 günlük tedavi sonrası hem NO düzeyi hem de arginaz aktivitesi düşerek sağlıklı gönüllü kontrollerin seviyesine ulaştı ($p<0.001$). Başlangıç ve 30. gündeki plazma arginaz aktiviteleri ve NO düzeyleri arasında anlamlı bir ilişki saptanmadı.

Sonuç: Arginaz ve NO sentaz arasındaki ilişkinin sadece aynı substrat için yarışmadan ibaret olmadığı düşünülmüştür. Tedavi modaliteleri, depresif dönemin klinik olarak iyileşmesi ve diğer başka faktörler NO düzeylerinin ve arginaz aktivitesinin normalleşmesinde önemli olabilir.

Anahtar sözcükler: İki uçlu bozukluk, nitrik oksit, arginaz, depresyon, tedavi

Journal of Mood Disorders 2011;1:1-6

ABSTRACT:

Alterations in plasma nitric oxide level and arginase activity during the treatment of bipolar depressive episode

Objective: Several studies showed that nitric oxide (NO) plays role in the pathophysiology of bipolar disorder (BD). Arginase might be important in the regulation of NO since both arginase and NO synthase use L-arginine as a common substrate. Although studies generally found increased levels of NO in BD, the picture is less clear for arginase activity. The present study aimed to investigate arginase activity together with NO levels in patients with bipolar disorder-depressed episode (BD-DE) in a prospective design.

Method(s): 33 BD-DE patients, diagnosed according to DSM-IV, and 33 age, gender and smoking satus matched healthy volunteer controls were included. Serum NO levels and arginase activities of the patient group were measured before any treatment and compared to that of controls. The patients were then allowed for episode specific naturalistic treatments including pharmacotherapy and electroconvulsive therapy when indicated. NO and arginase levels were evaluated on the 30th day of treatment and clinical outcome was measured by the Hamilton Depression Scale.

Results: Pretreatment NO levels and arginase activities of the patients were significantly higher than the healthy volunteers ($p<0.001$ in both). After 30 days of treatment, both NO levels and arginase activities were decreased ($p<0.001$), and reached to the volunteers' levels. There was no significant correlation between plasma arginase and NO levels in either at the beginning or 30th day evaluations.

Conclusion(s): Interaction between arginase and NO synthase does not seem to be as simple as just competing for a common substrate. Treatment modalities, clinical improvement of the depressive episode or other factors might be important in the normalization of NO levels and arginase activity.

Key words: Bipolar disorder, nitric oxide, arginase, depressive episode, treatment

Journal of Mood Disorders 2011;1:1-6

[1]MD, Gaziantep University, Faculty of Medicine, Department of Biochemistry and Clinical Biochemistry, Gaziantep-Turkey
[2]MD, Associate Professor, Gaziantep University, Faculty of Medicine, Department of Biochemistry and Clinical Biochemistry, Gaziantep-Turkey
[3]MD, Istanbul University, Istanbul Faculty of Medicine, Department of Child and Adolescent Psychiatry, Istanbul-Turkey
[4]MD, Nizip State Hospital, Psychiatry Clinic, Gaziantep-Turkey
[5]MD, Professor, Gaziantep University, Faculty of Medicine, Department of Psychiatry, Gaziantep-Turkey

Yazışma Adresi / Address reprint requests to: Ayşe Kılınçaslan MD, Istanbul University, Istanbul Faculty of Medicine, Department of Child and Adolescent Psychiatry, Istanbul-Turkey

Telefon / Phone: +90-212-414-2000

Elektronik posta adresi / E-mail address: ayse.ka@windowslive.com

Kabul tarihi / Date of acceptance: 1 Mart 2011 / March 1, 2011

Bağıntı beyanı:
N.Ö.C., İ.G.Ç., A.K., F.B.: Yazarlar bu makale ile ilgili olarak herhangi bir çıkar çatışması bildirmemişlerdir.
H.A.S.: Çok sayıda ilaç firmasından bilimsel aktiviteleri karşılığı honorarium kabul etmiştir.

Declaration of interest:
N.Ö.C., İ.G.Ç., A.K., F.B.: The authors reported no conflict of interest related to this article.
H.A.S.: Received honoraria for scientific activities from various pharmaceutical companies.

INTRODUCTION

Bipolar disorder (BD) is a prevalent, chronic, severe and highly disabling psychiatric disorder (1). Although the etiopathogenesis of BD has not yet been clarified, recent biological studies predominantly focus on neuroimaging, genetic and biochemical aspects of the disease.

An emerging body of evidence suggests that oxidative stress, which is defined as "a disturbance in prooxidant-antioxidant balance in favour of the former leading to potential damage" (2), may be an important mediating factor in the etiology of BD. Nitric oxide (NO), superoxide and hydroxyl radicals are the predominant reactive oxygen species (ROS) generated in physiological processes. Under normal conditions, these agents are eliminated by cellular enzymatic and non-enzymatic antioxidant defence mechanisms. Superoxide dismutase, catalase and glutathione peroxidase are important enzymatic antioxidant mechanisms. If ROS are not effectively eliminated, they can cause oxidative cell injury, such as peroxidation of lipids (membranes and organelles), proteins (receptors and enzymes) and DNA (3). Several studies reported that the patients with BD have significant oxidative imbalance in several steps e.g. increased NO levels (4-8), decreased antioxidant enzymes (4-6,9-13), increased lipid peroxidation (4,6,9,11-13) and DNA damage (14).

NO is a soluble gas produced from L-arginine by the activity of the enzyme NO synthase (NOS), that is present in peripheric tissues and neurons. NO is a free oxygen radical and also serves as a neurotransmitter or a secondary messenger (15) and involved in numerous physiologic functions such as noradrenaline and dopamine release, memory, learning, regulation of cerebrovascular system, modulation of wakefulness, modulation of nociception, olfaction, food intake and drinking (16). As a free radical, NO reacts rapidly with superoxide radical to form peroxynitrite, which is a very reactive species. All but one study (10) found that NO level is increased in all phases of BD namely; manic episode (3,6,7) euthymic episode (5) and depressed episode (4) when compared to the healthy volunteers. Additionally, NO is related to the pathogenesis of depression (16-18).

Arginase (EC 3.5.3.1) is a manganese-containing enzyme which converts L-arginine to L-ornitine and urea. This enzyme has 2 isoenzymes; the first is Arginase I, which functions in the urea cycle, and is located primarily in the cytoplasm of the hepatocytes. The second isoenzyme, arginase II is located in mitochondria of the cells of several tissues such as brain, kidney, small intestine and mammary gland (19) and has been implicated in the regulation of the arginine and ornithine concentrations in the cell. Ornitine has also an important role in the production of glutamate, gamma aminobutyric acid and proline.

L-arginine is the substrate of both arginase and NOS. Reciprocal regulation of these two enzymes in L-arginine-metabolising pathways has been demonstrated in wound repair, chronic renal failure, rheumatoid arthritis and glomerulonephritis (20-23). Although increased NO levels is frequently reported in bipolar patients, there is paucity studies on arginase activity in this population. Yanık et al. (6) found significantly lower arginase activity and increased NO levels in the patients with BD at the manic episode. Two other studies reported increased arginase activity in depression. While one found no relationship between connection with arginine and NO (24), the other did not assess the NO levels (25).

In this study we aimed to evaluate the course of NO levels and arginase activity in bipolar patients in depressive episode and compare the patients before and after treatment and to the healthy voluntary controls.

MATERIALS AND METHOD

Subjects

Thirty-three bipolar I depressive (BD-DE) patients who were admitted to the Mood Disorders Unit of Gaziantep University Department of Psychiatry and 33 healthy volunteers were included in this study. The patients and healthy voluntary controls with a history of drug abuse, chronic systemic diseases such as diabetes mellitus, hypertension, severe head injury or seizure disorders and those with obesity were excluded. Written informed consent was obtained from all participants and approval of the study was given by the Ethics Committee of Gaziantep University, Faculty of Medicine. Patient and control groups were matched for age, sex and smoking status. The diagnosis of BD was established by two qualified psychiatrists independently according to DSM-IV (26), and the Turkish version of Hamilton Depression Inventory (HAM-D; 27) was administered to the patients at the beginning and 30th day of the treatment.

Treatments

The patients were allowed for episode specific naturalistic treatments including antidepressants, mood stabilizers, atypical and typical antipsychotics and electroconvulsive therapy (ECT) when indicated (Table 1).

Table 1: Treatment features applied to patients at the beginning (1st day) and 30th day

Outcome	Day 1	Day 30
HAM-D* (mean±sd)	28.2±2.6	6.2±1.3
Treatment	**n (%)**	**n (%)**
Electroconvulsive Therapy	-	17 (52%)
Atypical antipsychotics	-	9 (27%)
Classical antipsychotics	-	3 (9%)
Mood stabilizers	-	20 (60%)
Antidepressant	-	16 (48%)

HAM-D: Hamilton Depression Scale *p<0.001 paired sample T-test

Blood Sample Collection and Preparation

The subjects strictly refrained from alcohol or food intake and physical exercise after 08:00 pm on the day before blood sample collection. In the patient group, blood samples were collected twice during routine laboratory evaluation at 08:00 a.m. on the 1st (before episode specific treatment) and on the 30th day (after episode specific treatment) of the study. Blood was sampled only once from the healthy volunteers. The samples were collected into plain laboratory tubes which does not contain anticoagulants. The samples stand for 30 minutes for coagulation. After centrifugaton at 3000 rpm, for 10 min, plasma samples were obtained and stored at -80˚C.

Measurement of Plasma NO Level

The stable oxidation end products of NO, nitrite (NO_2) and nitrate (NO_3^-), as indicators of NO production were measured (28). For nitrite plus nitrate (total nitrite) detection, an aliquot of the sample was treated with copperized cadmium (Cd) in glycine buffer at pH 9.7 (2.5 to 3 g of Cd granules for a 4 mL reaction mixture) to reduce nitrate to nitrite and then mixed with fresh reagent and the absorbance was measured in a spectrophotometer (UV-1601 Shimadzu, Japan). Lineer regression was done using the peak areas from the nitrite standards. The resulting equation was then used to calculate the unknown sample concentrations. All chemicals used in this assay were obtained from Sigma except cadmium granules (Fluka). Results were expressed as micromoles per liter (µmol/L) at the first day (NO_1) and 30th day (NO_{30}).

Measurement of Plasma Arginase Activity

Plasma arginase activity was measured according to the method of Geyer and Dabich (1971) with some modification for plasma. Briefly, plasma was diluted 10 times with a solution of 5mmol/l Mn^{2+} and in-cubated for 8 min at 55°C. Then, 0.2 preincubated plasma, 0.4ml 25mmol/l L-arginine, and 0.4ml 40mmol/l carbonate tampon (pH: 9.7) were incubated for 60 min at 37°C. After incubation, the reaction was stopped and the sample was deproteinized by adding 1ml of 0.5ml 1 N HClO4. The urea level was measured spectrophotometrically through the method of thiosemicarbazide-diacetylmonoxime- urea in the supernatants obtained by the centrifugation of the tubes for 5 min at 5000 rpm. A zero time blank which was not incubated and of which the ingredient was the same as the experiment tube in which enzyme activity was measured was used. Plasma arginase activity is expressed as unit. One unit plasma arginase was defined as the enzyme activity that produces 1 µmol of urea per minute.

Statistical Analyses

SPSS 11.0 for Windows computing program was used for the statistical analyses. When conditions were satisfied parametric analysis was used. The significance of differences between groups was estimated by independent sample t-test and within groups was evaluated using paired-sample T test. Mann-Whitney U and chi-square tests were used for non-parametric between group differences and for proportions, respectively. Bivariate correlations were examined via Pearson correlation coefficients. Differences were accepted as significant at the level of $p<0.05$.

RESULTS

Both the patient and control groups were equally consisted of 13 females and 20 males. The mean, standard deviation and range of ages in both groups were 30.8, 9.7 and 19-55, respectively. Patients were presented according their HAM-D scores at the beginning (drug naive) and 30th day of their treatments (Table 1) along with their treatment features.

The mean pretreatment NO levels (NO_1) of the patients was higher than the healthy volunteers, but mean NO levels on the 30th day of treatment (NO_{30}) was

Table 2: NO levels and arginase activities in patients with BD-DE vs. healthy voluntary controls

	BD-DE (N=33)	Control (N=33)	P values
NO_1 (µmol/L)	192.2±13.7	109.5±6.2	p<0.001*
NO_{30} (µmol/L)	126.1±6.3		p=0.11**
$Arginase_1$ (U/mL)	17.5±1.8	8.5±1.5	p<0.001*
$Arginase_{30}$ (U/mL)	10.3±1.4		p=0.45*

Results were expressed as mean±standard deviation, *Mann-Whitney U test, **Independent sample T-test, BD-DE: Type I bipolar disorder depressive episode.
NO_1: Nitric oxide levels at the 1st day, NO_{30}: Nitric oxide levels at the 30th day
$Arginase_1$: Arginase activity at the 1st day, $Arginase_{30}$: Arginase activity at the 30th day

similar to each others (Table 2). When NO_1 and NO_{30} were compared within the patients, NO_1 levels were found significantly higher (p<0.001).

Arginase activity showed a similar trend; mean pretreatment arginase activity (Arg_1) of the patient group was higher than the control group, but arginase activity on the 30th day (Arg_{30}) fell down to the levels of the control group (Table 2). Also Arg_1 levels were significantly higher than Arg_{30} levels (p<0.01).

When the above groups were compared for each gender, the statitical significances did not change. There was no correlation between the plasma arginase activities and NO levels in either at the beginning or on the 30th day of evaluations (pearson correlation test, p>0.05).

DISCUSSION

NO is implicated in the pathophysiology of psychiatric disorders through its ability to modulate certain forms of synaptic plasticity and its capacity to be transformed to a highly active free radical (29). Increased levels of NO are reported in adult patients in the manic (6,7) and eutymic episodes (5). After these cross-sectional studies, Selek et al. and Gergerlioglu et al. investigated the course of NO levels in one month period in depressed and manic episodes, respectively. Selek et al. found that NO levels were higher than healthy voluntary controls, but normalised after 30 days, however, Gergerlioglu et al. reported that NO levels stayed high during treatment. These latter studies investigated the groups while the groups were on variety of treatments at both time points (1st and 30th days) and speculated that medication or ECT did not effect the NO levels. In our study, we followed BD-depressed patients in drug naive and after one-month treatment phases in other to lessen the confounding effect of medication on the enzyme and NO levels.

Concerning the studies which investigated arginase levels in patients with mood disorders, Szilagyi in her pioneer study on patients with BD, found a phase dependent change in serum arginase activity, that is normal in manic phases, higher in symptom free intervals and highest during depression (24). Elgun and Kumbasar found increased serum arginase activity in drug-naive depressed patients and, patients with major depression had higher levels than those with minor depression (25).

Due to the close relatonship between NO synthase and arginase activities as probable competing enzymes for the same substrate, it is wise to evaluate these two in the same patient group. Such a study was conducted on BD patients in the manic phase, and found increased NO levels and decreased arginase activities (6). All the patients in this study were evaluated while they were taking stable doses of mood stabilizers, and typical or atypical antipsychotics. Although the authors claim that basic results of their study may not be effected by medications, they admit this as a limitation. In fact, drugs were demonstrated to alter NO levels in animal studies (30,31). Also our study was conducted with patients in the depressed phase, which may suggest that arginase activitiy might be differently regulated in mania and depression. This claim has also been supported by the findings of Szilagyi, as well as Elgun and Kumbasar (24,25).

Our study has several important characteristics that former studies did not show: Patients with BD-depressive episode are first to be evaluated in the treatment naive (mediaction or ECT) phase. The other one is evaluating NO levels and arginase activities together in this patient group. The third one is its longutidinal design which allowed us to observe the course of NO levels and arginase activities with treatment. One of the limitations of our study is the application of different treatment modalities, including ECT and different classes of medications, rather than a specific treatment. Also, patients with comorbid psychiatric conditions like anxiety disorders have not been excluded from the study as comorbid disorders are very prevalent in BD.

Patients with BD-DE were confirmed to have increased levels of NO and arginase activities compared to healthy volunteers apart from treatment effects. In this phase, arginase activities and NO levels were both increased, which suggests that the interaction between arginase and NO synthase are not as simple as just competing for a

common substrate. Additionally there is a possibility of differing regulation mechanisms in mania and depression. After clinical improvement with treatment, both NO and enzyme levels decreased and reached to that of healthy voluntary controls. It is difficult to determine either improvement in symptoms and coming to the euthymic phase, or the treatment modalities (ECT, antidepressants, mood stabilizers, atypical and typical antipsychotics) have mainly caused this change. Further studies investigating the specific effects of these treatment modalities on arginase and NO levels should be conducted.

References:

1. Belmaker RH. Medical progress: bipolar disorder. New England Journal of Medicine. 2004;51:476-86.
2. Sies H. Oxidative stress: from basic research to clinical application. Am J Med. 1991;91:31S–38S.
3. Andreazza AC, Kauer-Sant'anna M, Frey BN, Bond DJ, Kapczinski F, Young LT, et al. Oxidative stress markers in bipolar disorder: a meta-analysis. J Affect Disord. 2008;111:135-44.
4. Gergerlioglu HS, Savas HA, Bulbul F, Selek S, Uz E, Yumru M. Changes in nitric oxide level and superoxide dismutase activity during antimanic treatment. Prog Neuropsychopharmacol Biol Psychiatry. 2007;31:697–702.
5. Selek S, Savas HA, Gergerlioglu HS, Bulbul F, Uz E, Yumru M. The course of nitric oxide and superoxide dismutase during treatment of bipolar depressive episode. J Affect Disord. 2008;107:89–94.
6. Savas HA, Gergerlioglu HS, Armutcu F. Elevated serum nitric oxide and superoxide dismutase in euthymic bipolar patients: impact of past episodes. World J Biol Psychiatry. 2006;7:51–5.
7. Yanik M, Vural H, Tutkun H, Zoroglu SS, Savas HA, Herken H, et al. The role of the arginine-nitric oxide pathway in the pathogenesis of bipolar affective disorder. Eur Arch Psychiatry Clin Neurosci. 2004;254:43–7.
8. Savas HA, Herken H, Yurekli M, Uz E, Tutkun H, Zoroglu SS, et al. Possible role of nitric oxide and adrenomedullin in bipolar affective disorder. Neuropsychobiology. 2002;45:57–61.
9. Andreazza AC, Cassini C, Rosa AR, Leite MC, de Almeida LM, Nardin P, et al. Serum S100B and antioxidant enzymes in bipolar patients. J Psychiatr Res. 2007;41:523–29.
10. Machado-Vieira R, Andreazza AC, Viale CI, Zanatto V, Cereser Jr V, da Silva Vargas R, et al. Oxidative stress parameters in unmedicated and treated bipolar subjects during initial manic episode: a possible role for lithium antioxidant effects. Neurosci Lett. 2007;421:33–6.
11. Ozcan ME, Gulec M, Ozerol E, Polat R, Akyol O. Antioxidant enzyme activities and oxidative stress in affective disorders. Int Clin Psychopharmacol. 2004;19:89–95.
12. Ranjekar PK, Hinge A, Hegde MV, Ghate M, Kale A, Sitasawad S, et al. Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. Psychiatry Res. 2003;121:109–22.
13. Kuloglu M, Ustundag B, Atmaca M, Canatan H, Tezcan AE, Cinkilinc N. Lipid peroxidation and antioxidant enzyme levels in patients with schizophrenia and bipolar disorder. Cell Biochem Funct. 2002;20:171-5.
14. Andreazza AC, Frey BN, Erdtmann B, Salvador M, Goncalves CA, Kapczinski F. DNA damage in bipolar disorder. Psychiatry Res. 2007;153:27–32.
15. Schulman H. Nitric oxide: a spatial second messenger. Mol Psychiatry. 1997;2:296-9.
16. Papageorgiou C, Grapsa E, Zerefos N, Stamatelopoulos S, Christodoulou GN. Association of serum nitric oxide levels with depressive symptoms: a study with end-stage renal failure patients. Psychother Psychosom. 2001;70: 216–20.
17. Suzuki E, Yagi G, Nakaki T, Kanba S, Asai M. Elevated plasma nitrate levels in depressive states. J Affect Disord. 2001;63:221–4
18. Srivastava N, Barthwal MK, Dalal PK, Agarwal AK, Nag D, Seth PK, et al. A study on nitric oxide, ß- adrenergic receptors and antioxidant status in the polymorphonuclear leukocytes from the patients of depression. J Affect Disord. 2002;72:45–52.
19. Jenkinson CP, Grody WW, Cederbaum SD. Comparative properties of arginases. Comp Biochem Physiol B Biochem Mol Biol. 1996;114:107–32.
20. Albina JE, Mills CD, Henry WL, Caldwell MD. Temporal expression of different pathways of L-arginine metabolism in healing wounds. J Immunol. 1990;144:3877–80.
21. Huang LW, Chang KL, Chen CJ, Liu HW. Arginase levels are increased in patients with rheumatoid arthritis. Kaohsiung J Med Sci. 2001;17:358–63.
22. Durak I, Ozturk HS, Elgun S, Cimen MYB, Yalcyn S. Erythrocyte nitric oxide metabolism in patients with chronic renal failure. Clin Nephrol. 2001;55:460–4.
23. Jansen A, Lewis S, Cattell V, Cook HT. Arginase is a major pathway of L-arginine metabolism in nephritic glomeruli. Kidney Int. 1992;42:1107–12.
24. Szilagyi K. Serum arginase-activity in manic-depressive illness. Arch Psychiatr Nervenkr. 1971;214:390-8.
25. Elgun S, Kumbasar H. Increased serum arginase activity in depressed patients. Prog Neuro Psychopharmacol. Biol Psychiatry. 2000;24:227–32.
26. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th edition. Washington DC: DSM-IV-TR; 2004.
27. Akdemir A, Orsel S, Dağ I, Turkçapar H, Iscan N, Ozbay H. Hamilton depresyon derecelendirme ölçeği (HDDÖ)'nin geçerliliği, güvenilirliği ve klinikte kullanımı. 3P Dergisi. 1996;4:251-9.

28. Koltuksuz U, Irmak MK, Karaman A, Uz E, Var A, Ozyurt H, et al. Testicular NO levels after unilateral testicular torsion/detorsion in rats pretreated with caffeinic acid phenethyl ester. Urol Res. 2000;28:360-3.

29. Silberg G, Ben-Shachar D, Navon R. Genetic analysis of nitric oxide synthase 1 variants in schizophrenia and bipolar disorder. Am J Med Genet B Neuropsychiatr Genet. 2010;5:1318-28.

30. Kowalski J, Labuzek K, Herman ZS. Flupentixol and trifluperidol reduce secretion of tumor necrosis factor-alpha and nitric oxide by rat microglial cells. Neurochem Int. 2003;43:173-8.

31. Wegener G, Volke V, Harvey BH, Rosenberg R. Local, but not systemic, administration of serotonergic antidepressants decreases hippocampal nitric oxide synthase activity. Brain Res. 2003;959:128-34.