



Revista Brasileira de Psiquiatria

RBPPsychiatry

Official Journal of the Brazilian Psychiatric Association
Volume 34 • Supplement 2 • October/2012



ARTICLE

Nitric oxide plasma/serum levels in patients with schizophrenia: A systematic review and meta-analysis

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Received on October 7, 2011; accepted on February 29, 2012.

DESCRIPTORS

Nitric Oxide;
Schizophrenia;
Psychosis;
Plasma levels;
Serum levels;
Meta-analysis;
Systematic review.

Abstract

For the last 40 years, schizophrenia has been considered to be the result primarily of a dysfunction in brain dopaminergic pathways. In this review, it is described and discussed findings concerning nitric oxide-mediated neurotransmission in schizophrenia. Studies were searched in PubMed, SciELO, and LILACS using the terms *schizophrenia and nitric oxide plasma levels* or *nitric oxide serum levels*, with no time limit. The reference lists of selected articles were also hand-searched for additional articles. From 15 potential reports, 10 were eligible to be included in the review and meta-analysis. These studies included a total of 505 patients with schizophrenia and 339 healthy volunteers. No significant difference was found between patients and healthy controls regarding total nitrite plasma/serum levels (effect size $g = 0.285$, 95%CI = -0.205 to 0.774, $p = 0.254$). However, when studies with patients under antipsychotic treatment were examined separately, there was a significant difference between patients and healthy volunteers (effect size $g = 0.663$, 95%CI = 0.365 to 0.961, $p < 0.001$), showing that patients under treatment have higher levels of plasma/serum nitric oxide than controls. These results suggest that antipsychotics increase nitric oxide plasma/serum levels and that the nitric oxide pathway would be a fertile target for the development of new treatments for patients with schizophrenia.

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doi:10.1016/j.rbp.2012.07.001

Introduction

Schizophrenia is a devastating disorder that occurs in about 1% of the population throughout life. Individuals are usually affected somewhere between the end of adolescence and the beginning of adult life and the course of schizophrenia is chronic and debilitating.¹

For the last 40 years, schizophrenia was believed to be primarily the result of a dysfunction in brain dopaminergic pathways.² However, antipsychotic drugs that act as dopamine receptor antagonists have proven effective in treating only some of the symptoms of schizophrenia.

Recent research suggests that hypoactivity of a subtype of glutamatergic receptor, the N-methyl-D-aspartate (NMDA) receptor, may be involved in schizophrenia.^{3,4} One of the pieces of evidence supporting this hypothesis comes from the psychotic syndrome produced by NMDA receptor antagonists. This pharmacological model is the one that best mimics schizophrenia symptoms, as it induces both the positive and negative symptoms commonly seen in the disorder.⁵⁻⁸ However, research on NMDA receptor agonists for the treatment of schizophrenia has produced inconsistent results⁹, possibly due to the development of rapid tolerance to these compounds secondary to down-regulation of NMDA receptors.¹⁰

Accordingly, researchers have suggested the possible implication of nitric oxide (NO) in the pathophysiology of schizophrenia and other psychiatry disorders, such as bipolar disorder and depression.^{11,12}

NO is a gas with a unique chemistry and has been shown to influence the release of neurotransmitters, learning, memory, and neurodevelopment. In addition, questions have been raised about whether NO plays an important role in the maturation of neurons and synaptogenesis. The activation of NMDA receptors by glutamate results in calcium influx into the cell, which binds to calmodulin and stimulates the neuronal nitric oxide synthase (nNOS) enzyme to produce NO in the nervous system. NO activates guanylate cyclase, which increases the levels of the second messenger cyclic GMP (cyclic guanosine monophosphate). This "NMDA-NO-cyclic GMP pathway" has been demonstrated to modulate the release of neurotransmitters such as glutamate and dopamine. Although the direction of abnormalities in this pathway is still under debate, it has been repeatedly implicated in schizophrenia, and perhaps the development of drugs that act downstream NMDA receptors - on NO for instance - could circumvent the problem of the down-regulation of those receptors.¹²

In this review we describe empirical studies available in the literature that analyzed the role of NO in schizophrenia by examining NO plasma/serum levels in patients diagnosed with the condition.

Methods

The search was performed in the PubMed, SciELO, and LILACS databases using the keywords *schizophrenia* and *nitric oxide plasma levels* or *nitric oxide serum levels*, with no time limit. The reference lists of selected articles were also hand-searched for additional articles.

In order to be included in the analysis, studies had to meet the following criteria: 1) be published in any language; 2) include patients with schizophrenia diagnosed according

to DSM-IV-TR criteria; 3) have a comparison group of healthy subjects; 4) measure total nitrite plasma (or serum) levels to indirectly assess NO plasma levels.

Since NO is a very labile molecule and has a short half-life, the detection of NO in its native form is very difficult.¹³ In aqueous solution, NO reacts with molecular oxygen and accumulates in plasma/serum as nitrite (NO-2) and nitrate (NO-3) ions. These ions can be measured in biological fluids and have been used as correlates of NO levels.¹⁴ In most studies, plasma/serum NO metabolite levels are represented as total nitrite (nitrite + nitrate) after nitrate conversion to nitrite.¹⁵

Among the studies analyzed in this review, 80% investigated differences in total nitrite *plasma* levels between schizophrenia patients and healthy volunteers, while the remaining 20% investigated differences in total nitrite *serum* levels between the two groups. Given the considerable agreement concerning the equivalence between serum and plasma concentrations of certain drugs and assuming that the same is true for NO,¹⁶ we decided to analyze studies using plasma and serum as a single group.

Four studies measured patients' total nitrite levels at two different times - before and after antipsychotic treatment.¹⁷⁻²⁰ In these cases, we opted to work with measures obtained before antipsychotic treatment to ensure equivalence with the results of the remaining studies.

In order to select potentially relevant studies, three independent reviewers (JPMO, SI, CT) examined the abstracts identified in the literature search using the previously defined inclusion criteria. Disagreement at any stage was resolved by consensus. The studies included in the review are summarized in Table 1, which also describes the sociodemographic characteristics of the samples in each study.

Statistical analyses were performed with STATA 10.1 (StataCorp, College Station, Texas) using the METAN command. For each study, odds ratios and 95% confidence intervals were calculated. We used a random effects model that weighted the studies according to their inverse variance and provided the odds ratio and the corresponding confidence interval.

The between-study variability among the population effect sizes, i.e. heterogeneity, was assessed formally by applying Cochran's q test for homogeneity²¹ and informally by assessing a sample size independent descriptive measure of inconsistency I^2 .²² The I^2 index describes the percentage of the total variability in a set of effect sizes due to true heterogeneity, that is, between-study variability.²³ For example, a meta-analysis with $I^2 = 0$ means that all variability in effect size estimates is due to sampling error within studies. On the other hand, a meta-analysis with $I^2 = 50$ means that half of the total variability among effect sizes is caused not by sampling errors, but by true heterogeneity between studies. Higgins *et al.*²² proposed a tentative classification of I^2 values with the purpose of helping to interpret magnitude. Thus, percentages of around 25% ($I^2 = 25$), 50% ($I^2 = 50$), and 75% ($I^2 = 75$) would indicate low, medium, and high heterogeneity, respectively.

Publication bias

It was used Egger's regression test, which is a formal method of assessing publication bias,²⁴ implemented with the STATA function METABIAS.

Meta-regression

The effects of mean duration of illness on total nitrite plasma levels were assessed in a random effects meta-regression model by using the METAREG²⁵ command in STATA. The default option using residual maximum likelihood (REML) was selected.

Sensitivity analysis

To test how robust the results were to variations in the meta-analysis methodology, we examined the effect of excluding the two studies which evaluated NO serum levels instead of NO plasma levels.^{17,26}

Results

The search yielded 15 matches, of which 10 fulfilled the inclusion criteria for this meta-analysis. One study was excluded due to the presence of subjects with less than six months of disease duration. Based on the diagnostic criteria of DSM-IV TR, at least six months of illness are required for schizophrenia to be diagnosed.²⁷ Four articles were excluded because they measured levels of individual NO metabolites rather than total nitrite levels.²⁸⁻³¹ Thus, 10 studies provided comparative data from patients versus controls (Table 1).

Among the 10 studies analyzed in this review, all investigated differences in total nitrite plasma/serum levels between schizophrenia patients and healthy volunteers. The levels of plasma/serum total nitrite ranged from $3.37 \pm 2.27 \mu\text{mol/L}$ ²⁰ to $69.2 \pm 13.2 \mu\text{mol/L}$ ³² in healthy volunteers and from $1.85 \pm 0.70 \mu\text{mol/L}$ ²⁰ to $102.8 \pm 34.7 \mu\text{mol/L}$ ³² in patients with schizophrenia.

As shown in Figure 1, no significant difference was found between patients and healthy controls regarding total

nitrite plasma/serum concentrations (effect size $g = 0.285$, $95\%CI = -0.205$ to 0.774 , $p = 0.254$). Between-study heterogeneity ($I^2 = 90.8\%$, $Q = 98.22$, $p < 0.001$) was found, but there was no evidence of publication bias ($p = 0.466$). The meta-regression analysis showed a significant moderating effect of mean duration of illness (7 studies; $r = 0.153$; $p = 0.005$).

When studies with drug-free patients were evaluated separately (Figure 2), no significant difference was found between patients and healthy controls regarding total nitrite plasma/serum concentrations (effect size $g = -0.109$, $95\%CI = -0.856$ to 0.637 , $p = 0.774$). There was between-study heterogeneity ($I^2 = 90.7\%$, $Q = 43.01$, $p < 0.001$), but no evidence of publication bias ($p = 0.066$). The meta-regression analysis showed no effect of duration of illness (4 studies; $r = 0.147$; $p = 0.057$).

However, when we examined only the studies with patients under treatment (Figure 3), there was a significant difference between patients and healthy volunteers (effect size $g = 0.663$, $95\%CI = 0.365$ to 0.961 , $p < 0.001$), showing that patients under antipsychotic drug treatment have higher levels of plasma/serum total nitrite than controls. A moderate between-study heterogeneity ($I^2 = 56.0\%$, $Q = 9.10$, $p = 0.059$) was found and there was no evidence of publication bias ($p = 0.819$). The meta-regression analyses showed no significant interactions.

All the results of this meta-analysis remained the same when we excluded the two studies which worked with NO serum levels:^{17,26} all studies (effect size $g = 0.209$, $95\%CI = -0.377$ to 0.795 , $p = 0.484$); drug-free studies (effect size $g = -0.378$, $95\%CI = -1.071$ to 0.316 , $p = 0.286$); and under-treatment studies (effect size $g = 0.765$, $95\%CI = 0.482$ to 1.048 , $p = 0.000$).

Table 1 - Studies included in the meta-analysis

Reference	Subjects	N (M/F)	Duration of illness (years)	Antipsychotics	Mean age \pm SD (years)	Age at onset \pm SD (years)
Atmaca <i>et al.</i> ¹⁸	SZ	21 (14/7)	ND	No	30.4 \pm 7.8	ND
	CL	21 (14/7)			29.2 \pm 6.7	
Taneli <i>et al.</i> ¹⁷	SZ	20 (4/16)	1.2 \pm 1.0	No	26.8 \pm 8.5	23.4 \pm 2.9
	CL	20 (13/7)			25.5 \pm 5.8	
Akyol <i>et al.</i> ³⁶	SZ	100 (68/32)	10.0 \pm 6.9	Yes	34.29 \pm 9.39	24.3 \pm 7.2
	CL	51 (35/16)			35.49 \pm 10.69	
Zoroglu <i>et al.</i> ³⁷	SZ	82 (59/23)	12.1 \pm 8.9	Yes	33.64 \pm 11.08	ND
	CL	21 (15/6)			33.53 \pm 10.92	
Nakano <i>et al.</i> ²⁰	SZ	30 (18/12)	1.9 \pm 0.7	No	38.0 \pm 15.0	ND
	CL	30 (18/12)			41.0 \pm 19.0	
Yilmaz <i>et al.</i> ²⁶	SZ	66 (66/0)	12.1 \pm 10.0	Yes	33.6 \pm 11.0	22 \pm 9
	CL	28 (28/0)			34.4 \pm 12.0	
Lee <i>et al.</i> ¹⁹	SZ	55 (24/31)	5.6 \pm 5.2	DN	33.6 \pm 9.9	27.5 \pm 9.5
	CL	55 (24/31)			33.5 \pm 9.9	
Yanik <i>et al.</i> ³⁸	SZ	46 (36/10)	ND	Yes	34.0 \pm 11.8	ND
	CL	32 (22/10)			32.9 \pm 13.1	
Kim <i>et al.</i> ³⁹	SZ	45 (45/0)	4.3 \pm 5.4	DN	30.3 \pm 8.5	26.5 \pm 8.4
	CL	45 (45/0)			30.5 \pm 8.2	
Djordjević <i>et al.</i> ³³	SZ	40 (24/16)	ND	Yes	30.2 \pm 7.9	ND
	CL	36 (22/14)			29.8 \pm 5.8	

CL: controls; DN: Drug Naïve; F: female; M: male; N: number of subjects; ND: not described; SZ: schizophrenics.

Discussion

The neurobiology of schizophrenia is characterized by abnormalities in neurotransmission pathways including glutamate, GABA, and dopamine.^{3,33} The activation of glutamatergic NMDA receptors results in calcium influx into the cell, which stimulates nNOS to produce NO, which in turn activates guanylate cyclase resulting in increased production of cyclic

GMP. Dysfunction in this “NMDA-NO-cyclicGMP” pathway has been implicated in schizophrenia.^{34,35}

Because the direction of abnormalities in this pathway is still under debate, in this meta-analysis we tried to better understand the role of NO in patients with schizophrenia by investigating whether there were differences in its plasma/serum concentrations (as reflected by total nitrite) between subjects with schizophrenia and healthy controls.

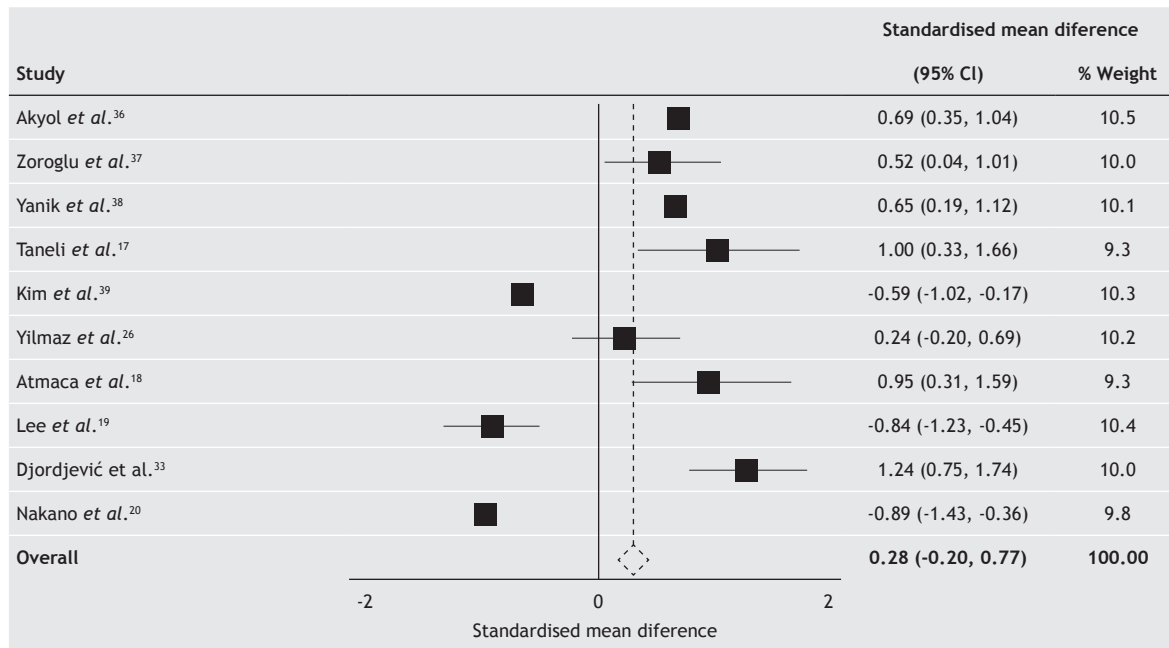


Figure 1 - Comparison of plasma/serum levels of total nitrite between schizophrenic patients and healthy controls in the 10 studies under investigation.

No significant difference was found between patients and healthy controls (effect size $g = 0.285$, 95%CI = -0.205 to 0.774, $p = 0.254$). Between-study heterogeneity was found ($I^2 = 90.8\%$, $Q = 98.22$, $p < 0.001$), but there was no evidence of publication bias ($p = 0.466$). The meta-regression analysis showed a significant moderating effect of mean duration of illness (7 studies; $r = 0.153$; $p = 0.005$).

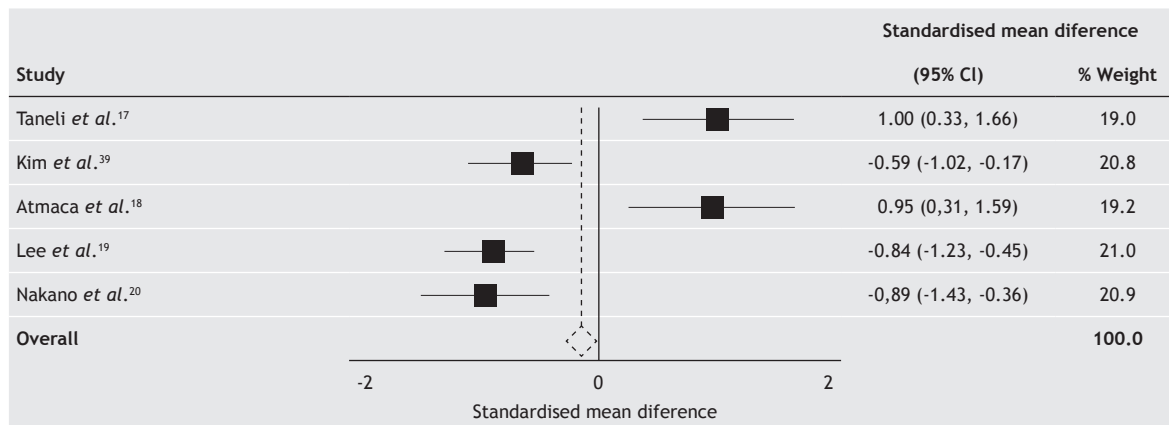


Figure 2 - Comparison of plasma/serum levels of total nitrite between drug-free schizophrenic patients and healthy controls. No significant difference was found between patients and healthy controls (effect size $g = -0.109$, 95% CI = -0.856 to 0.637, $p = 0.774$). Between-study heterogeneity was found ($I^2 = 90.7\%$, $Q = 43.01$, $p < 0.001$), but there was no evidence of publication bias ($p = 0.066$). The meta-regression analysis showed no effect of duration of illness (4 studies; $r = 0.147$; $p = 0.057$).

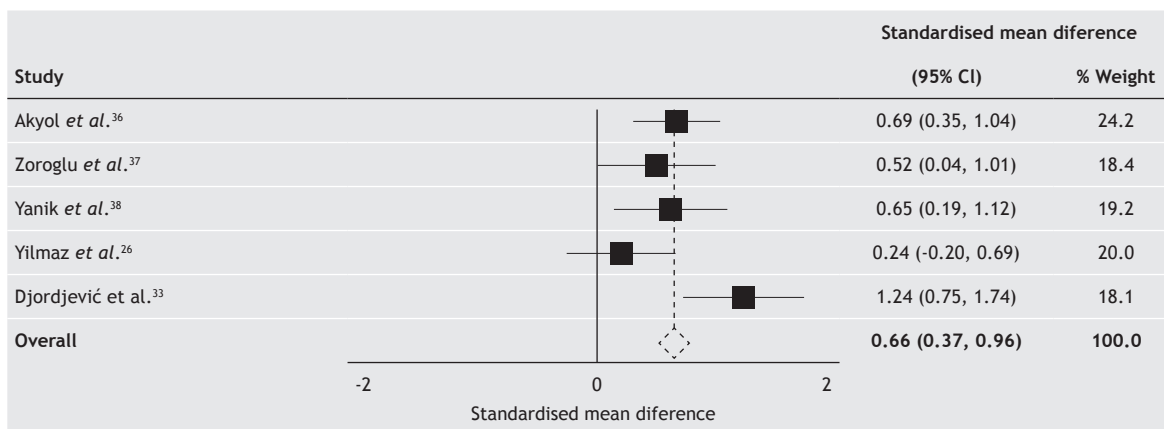


Figure 3 - Comparison of plasma/serum levels of total nitrite between schizophrenic patients receiving antipsychotics and healthy controls.

A significant difference between patients and healthy volunteers was found (effect size $g = 0.663$, 95%CI = 0.365 to 0.961, $p < 0.001$), showing that patients under medication treatment have higher levels of plasma NO than controls. A moderate between-study heterogeneity ($I^2 = 56.0\%$, $Q = 9.10$, $p = 0.059$) and no evidence of publication bias ($p = 0.819$) were found. The meta-regression analyses showed no significant interactions.

Among the 10 selected studies, there were no significant differences between patient groups and control groups regarding total nitrite plasma/serum levels. This finding may be explained by the presence of several studies with apparently contradictory results. Seven out of the 10 manuscripts found increased total nitrite levels in patients;^{17,18,26,32,36-38} while the remaining three found the opposite.^{1,9,20,39} Interestingly, all of these studies showed significant results. This is probably the reason why we detected between-study heterogeneity. Methodological variations across the studies may be the explanation for this discrepancy. For example, the duration of disease could be a relevant factor for the levels of NO, given the possible adaptive mechanisms related to the evolution of symptoms. Seven out of 10 studies made reference to the duration of disease in the patient group and the meta-regression analysis showed a significant moderating effect of mean duration of illness, showing a positive correlation between the latter and levels of NO. In this regard, there are two studies in the literature that measured NO metabolite levels during the first episode of schizophrenia. In 1996, Das *et al.* found low plasma nitrate levels in first-episode patients, and in 2004 Ramirez *et al.*⁴⁰ reported low concentrations of nitrite and nitrate in the cerebrospinal fluid of first-episode patients.^{28,40} Studies with first-episode patients and a longitudinal design are likely to foster a greater understanding of the relationship between duration of illness and NO levels. For example, studies that measure NO serum levels could generate correlations between NO levels and different phases of schizophrenia.

The use of antipsychotics by patients may influence the results of these studies. When the five studies with drug-free patients were evaluated, no significant difference was found between patients and healthy controls regarding total nitrite plasma/serum concentrations. However, when we examined only the five studies with patients under antipsychotic treatment, a significant difference between patients and healthy

volunteers was found, showing that patients taking antipsychotics have higher levels of plasma/serum NO than controls. In 1996, Das *et al.* found decreased plasma nitrate levels in first-episode schizophrenic patients who were also drug naïve.²⁸ In addition, a study in which the patients spent a longer period without taking antipsychotic medication before the beginning of the trials (drug-free for at least four weeks) reported decreased total plasma nitrite levels in the patient group, and that six-week antipsychotic treatment with risperidone increased these levels in correlation with clinical response. Significant changes of nitrite plasma levels between baseline and the end of the treatment in the patient group were found and compared between the 37 responders to treatment ($\geq 30\%$ improvement in PANSS score) and the 18 non-responders to treatment. Among responders, total plasma nitrite levels after treatment significantly increased when compared to baseline. However, there were no significant changes among non-responders. The authors suggested that the improvement of psychiatric symptoms can lead to partial normalization of a deficiency in NO after treatment.¹⁹ Finally, three articles reported an increase in cerebrospinal fluid levels of cyclic GMP in patients with schizophrenia after treatment with antipsychotic drugs.⁴¹⁻⁴³ As observed previously, the mechanism of action of NO involves increases in cyclic GMP concentrations through the activation of soluble guanylate cyclase.

Although plasma and serum are not the same thing and the decision to analyze studies that quantified NO serum levels and NO plasma levels together could be regarded as generating a potential confounding factor in this study. Nonetheless, the sensitivity analyses indicated that this decision did not affect the main results of this meta-analysis. All the results of the meta-analysis remained the same when the two studies that evaluated NO serum levels were excluded.

It is essential that future studies be more careful in controlling for potential confounding factors that are known to be relevant in schizophrenia. In this regard, well-designed studies

must take into account variables such as disease duration and staging, schizophrenia subtype, tobacco use, and antipsychotic treatment.⁴⁴ Also, further studies should examine potential correlations between the intensity and quality of symptoms (as measured by scales like the PANSS), stage of disease, and plasma/serum levels of NO in schizophrenia patients.

Conclusion

High between-study heterogeneity was found and this could be responsible for the meta-analysis finding of no significant differences between the patient group and the control group regarding total nitrite plasma/serum levels. There were studies with apparently contradictory results and they could be divided into two groups: one group suggesting increased plasma/serum levels of total nitrites in patients when compared with healthy volunteers and another one supporting a possible decrease in such levels. It is thus likely that the results of one group were cancelled out by the results of the other.

One of the findings of this review was a positive correlation between the duration of disease and levels of NO. In other words, the longer the duration of the disease is, the greater the NO levels in patients. We speculate that this could be explained both by pathophysiological differences between the acute and chronic phases of schizophrenia as well as by the use of medications along the course of the disease.

Lastly, patients under antipsychotic treatment were found to have higher levels of NO metabolites than controls. This finding might be related to a possible stimulation of the nitric pathway by these drugs. It is believed that this pathway constitutes a fertile target for the development of new treatments for patients with schizophrenia.

Disclosures

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* Modest

** Significant

*** Significant. Amounts given to the author's institution or to a colleague for research in which the author has participation, not directly to the author.

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