# OXIDATIVE STRESS IN SCHIZOPHRENIA - FOCUSING ON THE MAIN MARKERS

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## SUMMARY

*Oxidative stress is the condition arising from imbalance between toxic reactive oxygen species and antioxidant systems. It is believed that increased oxidative stress may be relevant to the pathophysiology of schizophrenia. In this way, the main markers of the lipid peroxidation processes include 4-hydroxynonenal and malondialdehyde. On the other side, the potential toxicity of free radicals is counteracted by a number of cytoprotective antioxidant enzymes that limit the damage, such as superoxide dismutase and glutathione peroxidase.*

*However, the reports regarding the status of oxidative stress markers schizophrenia are very inconsistent, with various authors stating both increased and decreased activities of the main antioxidant enzymes, while others did not observe any significant modifications, as compared to control groups. Similar aspects were also reported in the case of the lipid peroxidation markers, although in here the contradictions are much more reduced than in the case of the antioxidant defences. It is generally believed that the equivocal results mentioned above may be due to different tissues studies, different species or the administrated treatment and the duration of the disease/treatment.*

*In this context, in the present paper we were interested to review some studies regarding the oxidative stress status in patients and animal models of schizophrenia, by referring mainly to antioxidant enzymes and lipid peroxidation markers.*

**Key words:** *schizophrenia - oxidative stress - 4-hydroxynonenal – malondialdehyde - superoxide dismutase - glutathione peroxidase*

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## INTRODUCTION

Oxidative stress is the condition arising from imbalance between toxic reactive oxygen species (ROS) and antioxidant systems. Various tissues have different susceptibilities to oxidative stress. Brain is particularly more vulnerable to oxidative damage due to relatively low levels of antioxidants, high levels of polyunsaturated fatty acids, high metal content and oxygen utilization (Smith 2006, Sultana et al. 2008, Hritcu et al. 2009).

Schizophrenia is a common psychiatric disorder, marked by gross distortion from reality; disturbances in thinking, feeling and behavior. It is believed that increased oxidative stress may be relevant to the pathophysiology of schizophrenia, but most of the results regarding this subject are contrasting (Kunz et al. 2008, Dadheech et al. 2008, Wood et al. 2009, Padurariu et al. 2010). Some of the lipoperoxidation metabolites include malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), which are considered the most specific and sensitive measures of lipid auto-oxidation (Haliwel et al. 2007, Zarkovic 2003). The potential toxicity of free radicals is counteracted by a number of cytoprotective enzymes and antioxidants that limit the damage. This protective mechanism function cooperatively in the form of a cascade which includes various antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) (Dadheech et al. 2008, Ciobica et al. 2009)

Considering that schizophrenia is the neuro-psychiatric disorder with the most contrasting evidences regarding oxidative stress implication, in comparison with other disorders like Alzheimer's disease (which is characterized by a general reduction of the enzymatic antioxidant defense system and an increased of lipid peroxidation processes) (Baldeiras et al. 2008, Greilberger et al. 2008) or Parkinson's disease (where free radicals are leading to oxidative damage of dopaminergic neurons in the substantia nigra, increased lipid peroxidation and a decrease in glutathione concentration) (Seet et al. 2010, Ciobica et al. 2011), and based on our previous experience regarding the implication of oxidative stress in other neuropsychiatric diseases like mild cognitive impairment, Alzheimer's disease (Padurariu et al. 2010a), Parkinson's disease (Hritcu et al. 2008), anxiety (Ciobica et al. 2010, 2011), sleep or depression disorders (Vitalaru et al. 2010), in this mini-review we will try to summarize the most important aspects and discrepancies, regarding some oxidative stress markers in schizophrenia, by referring mainly to antioxidant enzymes modifications and lipid peroxidation markers, but also a few aspects regarding animal models and possible antioxidant therapy.

## METHODOLOGY

Studies were searched in the main scientific databases (e.g. Pubmed, Sciencedirect, Scopus, Google Scholar), until 26 July 2011, by using the following keywords "oxidative stress schizophrenia", "superoxide dismutase schizophrenia", "glutathione peroxidase schizophrenia", "4-hydroxynonenal schizophrenia" and "malondialdehyde schizophrenia". Cross-references for these key words were also counted in.

## ANTIOXIDANT ENZYMES

The production of reactive species is a core part of mitochondrial energy generation, and these species are dealt with by the body in multiple ways. These include SOD, that catalyses the conversion of superoxide radicals to hydrogen peroxide, which is then converted into water by GPX and catalase (Figure 1).
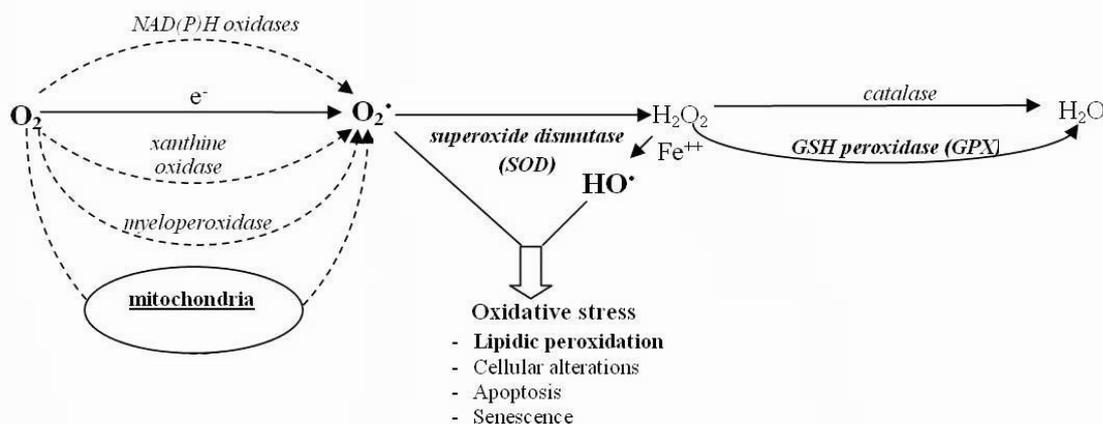


$O_2{-}\bullet$ = superoxide radical, $H_2O_2$ = hydrogen peroxide, $\bullet OH$= hydroxyl radical

**Figure 1.** Production of reactive oxygen species and the enzymatic defense mechanism against oxidative stress damage (Haulica et al. 2000)

While there are strong links between oxidative stress anomalies and the pathophysiology of schizophrenia, in vivo measurement of free radical concentrations is impractical because their reactive nature results in short half-lives and low levels. Oxidative status in clinical populations are typically assessed in other ways, such as the measurement of oxidative defences, particularly key enzymes we mentioned above (e.g. SOD, GPX, catalase), completed by assessment of the consequences of oxidative stress such as plasma lipid peroxides (Wood et al. 2009).

Studies performed in patients with schizophrenia have generally suggested the presence of a compromised antioxidant system (Pavlovic et al. 2002, Gysin et al. 2007, Dadheech et al. 2008, Wood et al. 2009), but this is not always consistent with specific observed parameters, which on the whole, showed evidences of dysregulation. In this way, for SOD, the first line of defense against ROS, both decreased (Ranjekar et al. 2003, Zhang et al. 2010) and increased (Reddy et al. 1991, Zhang et al. 2003) specific activities were reported. Moreover, studies stating no changes in SOD activity of patients with schizophrenia are mentioned (Yao et al. 1998). Similar contrasting aspects are also described for GPX (Herken et al. 2001, Gawryluk et al. 2011) or catalase, with results presenting reduced (Reddy et al. 1991) or increased (Herken et al. 2001) levels in patients with schizo-

phrenia, compared to control groups. In this way, increased antioxidant activity may reflect a preceding cellular oxidative stress or serve as a compensatory mechanism (Kuloglu et al. 2002, Dakhale et al. 2004, Rukimi et al. 2004, Kunz et al. 2008). Probably, this difference in various results can be attributed to different clinical symptoms, therapeutic features or duration of the illness.

In addition, in a recent paper published by Pazvantoglu et al. (2009), it was demonstrated that the severity of symptoms was associated with the decreased antioxidant level. Various antioxidants have been found to be related to negative symptoms, poor premorbid functions and computed tomography abnormalities. However, no significant relationship between duration of the disease and antioxidant levels was found. Also, antioxidants were reported to be different between various subtypes of schizophrenia: SOD and GPX activities were significantly lower in paranoid and residual subtypes, compared to both disorganized subtype and the control groups (Pazvantoglu et al. 2009).

Also, our group previously reported contrasting results regarding the antioxidant enzymes, with a significant increase of SOD specific activity and a decrease of GPX activity in patients with schizophrenia, compared to a control group (Padurariu et al. 2010b).

**Table 1.** The status of the main oxidative stress markers in some schizophrenia studies

| Study | Oxidative stress marker | Status (vs. control, unless otherwise stated) |
|---|---|---|
| Dadheech et al. 2008 | SOD | decreased activity in the red cells of never-treated schizophrenic patients |
| | GPX | decreased activity in the red cells of never-treated schizophrenic patients |
| | MDA | increased concentration in the blood of never-treated schizophrenic patients |
| Gama et al. 2006 | SOD | increased activity in the serum of schizophrenic patients in partial psychotic symptoms remission |
| | | no significant modifications in the serum as a result of haloperidol vs. clozapine treatment |
| | TBARS | increased concentration in the serum of schizophrenic patients in partial psychotic symptoms remission |
| | | increased levels in in the serum of patients treated with clozapine as compared to those treated with haloperidol |
| Gama et al. 2008 | SOD | no significant modifications in the serum among different subtypes (paranoid, disorganized, undifferentiated) |
| | | no significant modifications in the serum between different clinical course pattern (partial remission, marked symptoms and deteriorated) |
| | TBARS | no significant modifications in the serum among different subtypes (paranoid, disorganized, undifferentiated) |
| | | increased at the serum level in the subgroup with marked symptoms vs. deteriorated group |
| Kunz et al. 2008 | SOD | increased activity in the serum of treated schizophrenic patients |
| | TBARS | increased activity in the serum of treated schizophrenic patients |
| Martins et al. 2008 (in rats) | TBARS | increased concentration in the striatum after haloperidol treatment |
| | | decreased concentration in the prefrontal cortex after olanzapine and aripiprazole treatment |
| | | decreased levels at the cerebral cortex level after haloperidol, clozapine, olanzapine and aripiprazole administration |
| | | no significant modifications in the hippocampus levels as a result of haloperidol, clozapine, olanzapine and aripiprazole administration |
| Medina-Hernandez et al. 2007 | MDA + 4-HNE | increased concentrations in the serum of schizophrenic patients |
| | | increased concentration in the serum of treatment refractory schizophrenics, as compared to non-refractory schizophrenics |
| Miljevic et al. 2010 | SOD | increased activity in the plasma of schizophrenic patients chronically treated with clozapine |
| | GPX | decreased activity in the plasma of schizophrenic patients chronically treated with clozapine |
| | CAT | no significant modifications in the plasma of schizophrenic patients chronically treated with clozapine |
| Padurariu et al. 2010 | SOD | increased activity in the serum of schizophrenic patients of paranoid subtype |
| | | increased activity in the serum of schizophrenic patients treated with haloperidol and quetiapine |
| | GPX | decreased activity in the serum of schizophrenic patients of paranoid subtype |
| | | decreased activity in the serum of schizophrenic patients treated with haloperidol and risperidone |
| | MDA | increased levels in the serum of schizophrenic patients of paranoid subtype |
| | | increased levels in the serum of schizophrenic patients treated with haloperidol, quetiapine, olanzapine and risperidone |

| Study | Oxidative stress marker | Status (vs. control, unless otherwise stated) |
|---|---|---|
| Pazvantoglu et al. 2009 | TAOP | no significant differences in the serum of schizophrenic patients free of treatment for at least 2 weeks |
| | TPEROX | no significant differences in the serum of schizophrenic patients free of treatment for at least 2 weeks |
| Pavlovic et al. 2002 | SOD | increased activity in the red cells of schizophrenic patients with positive symptoms |
| | | no significant modifications in the red cells of schizophrenic patients with negative symptoms |
| | GPX | decreased activity in the red cells of schizophrenic patients with both positive and negative symptoms |
| | MDA | no significant changes in the erythrocytes of schizophrenic patients with both positive and negative symptoms |
| Radonjic et al. 2010 (animal model – phencyclidine administration) | GPX | decreased at the hippocampus level |
| | MDA | increased at the hippocampus and thalamus level |
| Rafa et al. 2009 | SOD | decreased activity in the plasma of treated or untreated schizophrenic patients |
| | GPX | decreased activity in the plasma of treated schizophrenic patients |
| | | no significant differences vs. control in the plasma of untreated schizophrenic patients |
| Singh et al. 2008 | SOD | decreased activity in the serum of schizophrenic patients chronically treated with haloperidol |
| | TBARS | increased concentration in the serum of schizophrenic patients chronically treated with haloperidol |
| Wang et al. 2009 | 4-HNE | increased levels in anterior cingulated brain of schizophrenic patients |
| Zhang et al. 2006 | SOD | decreased activity in the plasma of chronic schizophrenic patients |
| | GPX | decreased activity in the plasma of chronic schizophrenic patients |
| | MDA | increased concentration in the plasma of chronic schizophrenic patients |
| | SOD and GPX | decreased activity in the plasma of schizophrenic patients with paranoid and residual subtype vs. disorganized subtype |
| | SOD, GPX and MDA | no significant modifications at the plasma level regarding the differential effects of typical vs. atypical antipsychotics |
| Zhang et al. 2009 | TRX | increased in the serum of never medicated first episode schizophrenic patients |
| | | no significant modifications in the serum of chronic medicated schizophrenic patients |

Legend: SOD=superoxide dismutase; GPX=glutathione peroxidase; MDA=malondialdehyde; TBARS=thiobarbituric acid reactive substances; 4-HNE=4-hydroxynonenal; CAT=catalase; TAOP=total antioxidant potentials; TPEROX=total peroxide levels; TRX = thioredoxin

## LIPID PEROXIDATION MARKERS

Estimating levels of reactive oxidative products provides a very useful strategy to determine the impact of oxidative stress. Lipid peroxidation is often assayed by measuring thiobarbituric acid reactive substances (TBARS). The end products of lipid peroxidation, such as MDA assessment, have been very widely used indices of oxidative stress in clinical studies (Padurariu et al. 2010a, Baldeiras et al. 2008, Greilberger et al. 2008). Also, one of the most important products of lipid oxidation is 4-HNE, which is a highly cytotoxic reactive α,β-aldehyde that is generated during various physiolo-

gical and pathophysiological conditions based on the production of ROS (Schaur et al. 2003, Siems et al. 2003). Besides its important implications in Alzheimer's disease (where it can damage the cholinergic neurons by membrane permeabilization and apoptosis or cause impaired glutamate and glucose transport) (Negre-Salvayre et al. 2010) or Parkinson's disease (it is found in the Lewy bodies and mitochondria of PD patients) (Zarkovic 2003), 4-HNE seems to exert important actions also in schizophrenia, considering that increased levels of 4-HNE were found in patients with schizophrenia, as compared to normal (significantly increased by more then 47% vs. controls in the study of

Wang et al. from 2009), while Medina-Hernández et al. (2007) demonstrated an increase of 4-HNE concentration in treatment-refractory schizophrenics, as compared to non-refractory schizophrenics or with healthy controls, and also a significant correlation between the increased lipoperoxidation processes and delusions or emotional withdrawal symptoms (Medina-Hernández et al. 2007). Additionally, in the study of Wang et al. the separated analysis of the non-treated patients with schizophrenia revealed also an increased of 4-HNE levels in these subjects, suggesting that the increased 4-HNE in schizophrenia is not a result of the treatment and could be extremely relevant in the schizophrenic pathology.

Also, elevated levels of MDA have been shown in plasma, erythrocytes, leucocytes and platelets of patients with schizophrenia (Kunz et al. 2008). We also demonstrated similar results in the serum of schizophrenic patients (Padurariu et al. 2010b).

Additionally, it is believed that a high level of TBARS is a sign of peroxidative injury to membrane phospholipids. Neuronal functioning is affected by this injury either by changes in membrane fluidity or by alterations in membrane receptors (Mahadik et al. 2001), which can cause neurotransmitter uptake, release impairment and even cell death (Gama et al. 2006).

Some authors demonstrated that the extent of lipid peroxidation is positively correlated with the severity of symptoms in never-medicated patients and inversely with the levels of membrane-essential polyunsaturated fatty acids (Wood et al. 2009). However, few studies reported lipid peroxidation only in rats chronically treated with haloperidol, but not in animals treated with risperidone, olanzapine or clozapine (Parikh et al. 2003).

As in the case of the antioxidant enzymes, these contrasting results may be due to differences in species used for each study (rats and humans), different tissues (brain and serum), therapeutic features or duration of the illness. We will insist immediately on these aspects, referring especially to the differences between untreated and treated patients with schizophrenia or the differences exerted by typical vs. atypical treatment on oxidative stress status.

## DIFFERENCES BETWEEN UNTREATED AND TREATED SCHIZOPHRENIC PATIENTS

As we mentioned earlier, some contrasting results were obtained regarding oxidative stress status in patients with schizophrenia. One of the responsible factors for this inconsistency could be the difference between untreated and treated schizophrenic patients. Generally, most of the aforementioned studies were performed on treated patients. In a very interesting study published in 2009, Raffa and colleagues studied these oxidative stress markers in neuroleptic-free schizophrenic patients (n=36), compared to healthy

(n=46), but also schizophrenic treated patients (n=52). They found that comparing to the healthy controls, the patients with schizophrenia showed significantly lower levels of SOD, catalase and reduced glutathione. Among the schizophrenic patients, the activities of the SOD and catalase were recorded to be significantly lower in untreated patients than in the treated ones, suggesting that a decrease in the glutathione levels and the activities of the antioxidant enzymes in patients diagnosed with schizophrenia is not related to neuroleptic treatment and could be considered as a biological indicator of the degree of severity for the symptoms of schizophrenia (Raffa et al. 2009). This contradicted the idea that oxidative stress in patients with schizophrenia could be exacerbated further by treating them with antipsychotics, which possess pro-oxidant properties, based mostly of some papers reporting some possible oxidative stress induced by typical antipsychotics treatment in both humans (Kropp et al. 2005) and rats (Parikh et al. 2003, Pillai et al. 2007).

The Raffa group results where also confirmed by another 2 recent studies, one lead by Dadeech et al., which used patients with schizophrenia that had never taken any treatment and had come for consultation for the first time (they found increased levels of MDA and decreased activities for both SOD and GPX) (Dadeech et al. 2008) and one that determined the levels of a novel oxidative stress marker, thioredoxin (TRX) in never-medicated first-episode schizophrenic patients, lead by Zhang and colleagues. They found that serum TRX is significantly increased in first-episode drug-naive schizophrenia patients and greater compared to chronic schizophrenic patients on antipsychotic medication. Moreover, TRX levels did not differ between chronic patients and controls (Zhang et al. 2009).

These results suggest that the oxidative stress markers could be used to indicate the degree for the severity of the disease in untreated patients with schizophrenia. Some other authors affirm that excessive free radical production or oxidative stress may not be directly associated with the presence of the schizophrenia, but with the subtypes and/or the severity of the disorder (Pazvantoglu et al. 2009).

However, another study from Zhang et al. reported an increase in the SOD levels in neuroleptic-free schizophrenic patients (Zhang et al. 2003). This could be explained by the short neuroleptic-free period the authors used (two weeks-time in which the status of being drug-free might not have been long enough to allow the antioxidant enzymes to revert to their normal levels).

## EFFECTS OF TYPICAL VS. ATYPICAL ANTIPSYCHOTIC TREATMENT ON OXIDATIVE STRESS

In addition to the differences observed in treated vs. untreated schizophrenics, there are also controversies

regarding the oxidative stress status in patients treated with typical vs. atypical antipsychotics.

Some of the authors reported that chronic administration of typical antipsychotic haloperidol, but none of some atypicals antipsychotic like risperidone, clozapine or olanzapine induces oxidative stress by decreasing the activity of antioxidant enzymes and cause membrane lipid peroxidation (Parikh et al. 2003). However, there are studies demonstrating a decreased level of lipid peroxidation in the cerebral cortex, as a result of haloperidol chronic administration (Martins et al. 2008), as well as significant reduction of GPX specific activity in the blood of long-term clozapine-treated schizophrenic patients (Miljevic et al. 2010). Others hypothesized that the increased oxidative stress observed in some clozapine-treated groups could be indicative of illness severity, since this drug is formally indicated for a specific group of patients with schizophrenia, whose symptoms are refractory to other neuroleptics (Gama et al. 2006).

However, in a very complex study by Zhang et al. in 2006, no significant differences in plasma MDA levels and SOD, GPX and catalse activities were found among almost 100 patients from three subgroups treated with clozapine, risperidone and typical antipsychotics. It seems that both typical and atypical antipsychotic drugs may, at least partially, normalize the abnormal free radical metabolism in schizophrenia without a significant difference in their effects. Taken together, it is likely that pharmacological mechanisms of typical and atypical antipsychotics may be different, but end point effects on oxidative stress could be the same.

Also, in one of our previous studies we reported no major differences between patients treated with typical vs. atypical antipsychotics (Padurariu et al. 2010b).

## ANIMAL MODELS

For further research in this area, animal models of schizophrenia have been described. These could be very helpful in order to increase the accuracy of studies regarding the involvement of oxidative stress in different aspects of schizophrenia.

Phencyclidine administration to rodents represents one of the most suitable animal models of schizophrenia. Phencyclidine has psychotomimetic properties and is capable of producing both positive and negative symptoms of schizophrenia, exacerbating existing psychoses in schizophrenics or inducing cognitive dysfunctions in healthy volunteers. In this way, Radonjić et al. reported alterations in SOD activity and in the levels of lipid peroxides in different central regions of rats, as a result of postnatal treatment with phencyclidine (Radonjić et al. 2010). Similar aspects regarding glutathione depletion in rats were also recently reported (Castagne et al. 2004, Cabungal et al. 2007, Dean et al. 2009).

In addition, animal models can be used for understanding the effects of antipsychotics long-term treatment on the expression of antioxidant enzymes and oxidative neural cell injury. This could be important for explaining the possible differential mechanisms underlying some clinical side effects, but also planning long term-use or switch over between antipsychotics in the management of schizophrenia (Pillai et al. 2007). Still, as schizophrenia is a complex disease, animal models can only serve as models to a certain extent.

## ANTIOXIDANT TREATMENT

Some authors also suggested that the use of antioxidants might provide an improvement in the treatment of schizophrenic patients. In this way, Zhang and colleagues demonstrated in two different studies from 2001 that adding a Ginkgo biloba extract (well known for its antioxidant effects-Mantle et al. 2003) to classical haloperidol treatment, results in enhancing the effectiveness of the antipsychotic and reduces some extrapyramidal side effects (Zhang et al. 2001a). Moreover, adding Ginkgo biloba extract also resulted in better scores in the Scales for the Assessment of Positive and Negative Symptoms and decreased SOD levels (Zhang et al. 2001b).

The use of essential polyunsaturated fatty acids has also been suggested, considering that dysregulation of membrane phospholipid metabolism exists throughout the body from the onset of psychosis in patients with schizophrenia (Mahadik et al. 2003). Reduced levels of membrane essential polyunsaturated fatty acids like arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid or docosahexaenoic acids and their association with psychopathology have been consistently reported in both chronic-medicated schizophrenic patients as well as in never-medicated patients soon after the first episode of psychosis (Arvindakshan et al. 2003). These compounds are highly enriched in the brain and are crucial for brain and behavioral development (Reddy et al. 2010). A phospholipid metabolic defect may already exist before the start of psychosis, even at the embryonic stages (Mahadik et al. 2003). Because these membrane phospholipids play a crucial role in the membrane receptor-mediated signal transduction of several neuro-transmitters and growth factors, their altered metabolism may contribute to the reported abnormal information processing in schizophrenia (Peet et al. 2001, Emsley et al. 2002, Bitanihirwe et al. 2011). In this way, some authors reported that the severity of symptoms seems to correlate with the membrane arachidonic acid and docosahexaenoic acid levels, which are influenced by patient's dietary intake and lifestyle (Joy et al. 2006, Freeman et al. 2006, Berger et al. 2007). In addition, a combination of eicosapentaenoic/docosahexaenoic acid and vitamin C/E resulted in a significant reduction of schizophrenia psychopathology, suggesting that

essential polyunsaturated fatty acids supplementation could represent a very effective treatment to improve the outcome of the disease for an extended period of time (Arvindakshan et al. 2003). Also, the use of alfa-tocopherol alone is mentioned in some studies, especially for treating tardive dyskinesia that may appear as a side effect of antipsychotics long-term use (Yao et al. 2001). Additionally, Amminger and colleagues published in 2010 a double-blind, placebo-controlled trial conducted for over 4 years in young people with ultra-high risk of psychotic disorder, in which they report a significant decrease in the rate of progression to psychotic disorder as a result of long-chain omega-3 fatty acids administration (Amminger et al. 2010).

The above findings provide further evidence for a role of free radical-mediated psychopathology in schizophrenia and its complications. However, despite being promising, these studies require independent replication.

## CONCLUSIONS

Oxidative stress seems to be a key component in the schizophrenia pathophysiology. It is generally believed that oxidative stress may constitute a central point where other factors of vulnerability meet and their interactions could play a decisive role in the schizophrenic pattern.

Considering that oxidative stress is a factor that can be corrected, future studies that would clearly identify the etiologic relation between antioxidant deficiencies and schizophrenia, may provide prophylactic treatments, as well as new treatment schemes in addition to available antipsychotic schemes. This also emphasizes the possible importance of nutrient antioxidant supplementation to support the enzymatic defense system.

However, despite the fact that the use of agents which modulate oxidative stress represents an exciting opportunity for schizophrenia prevention and treatment, future studies also need to carefully determine which antioxidants, at what dosages and in what combinations will have the greatest therapeutic benefit with the least risk, considering the importance of free radicals in many biological reactions.

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