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Journal of Neurology & Translational Neuroscience

Research Article

Variation of the REDOX Status in Patients with Primary Autism after Antioxidant Therapy

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Submitted: 11 October 2017
Accepted: 03 December 2017
Published: 05 December 2017
ISSN: 2333-7087

Keywords
- Primary autism
- Oxidative stress
- Markers
- Antioxidant therapy

Abstract

Oxidative stress plays an important role in the pathophysiology of autism. This research was carried out to know if the indicators of oxidative stress improved after a diet and antioxidant treatment in patients with primary autism. We studied 40 cases with primary autism treated at the Clinical Genetics National Reference Service in Juan Manuel Márquez Pediatric Hospital and Mayabeque Neurodevelopment Consultation during October 2014 to June 2016. Markers of oxidative damage and antioxidant defense were determined. Patients with altered oxidative stress were treated with diet and antioxidant therapy and the response to treatment was re-evaluated over time. A high percentage of patients improved the results of protein oxidation products ($p < 0{,}001$), and total peroxide values post-therapy. However malonylaldehyde results remained elevate after treatment. No significant results were found for the antioxidant defense markers. The results of this research suggest that the use of antioxidant therapy associated with the established diet would be adequate.

ABBREVIATIONS

ASDs: Autism Spectrum Disorders; ROS: Reactive Oxygen Species; LPO: Lipid Peroxidation; CNS: Central Nervous System; Redox: Oxidation Reduction; BHA: Hydroxyl Butylanisole; BHT: Butilhidroxitolueno; MDA: Malonyldialdehyde; AOPP: Advanced Products of Protein Oxidation; FOX: Oxidation Xylenol Orange (Peroxide Concentrations); SOD1: Superoxide Dismutase; CAT: Catalase; GSH: Glutathione (free protein thiols in plasma); GPx: Glutathione Peroxidase; SAM: S-adenosylmethionine; SNV: Single Nucleotide Variant

INTRODUCTION

Autism spectrum disorders (ASD) comprise a heterogeneous group of disorders, both in their etiology and in their clinical presentation. According to the criteria of the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association in its fifth edition (DSM-5), these correspond to persistent deficits in social communication and social interaction across multiple contexts, restricted, repetitive patterns of behavior, interests, or activities. The symptoms cause a clinically significant impairment in social, occupational or other important areas of current functioning and must be presented in the early period of development [1].

Despite theories and research aimed at identifying the causes of this disorder and the reason for its epidemiological increase, but are still unknown. Many authors define autism etiologically as multifactorial and heterogeneous. Environmental influence on the developing brain of the child, chemical and food intolerances, neurotoxins and other factors are present and it would manifests in a worsening of autistic behaviors. That evidence a dysfunctional, stressed or hyperreactive immune system [2,3].

Oxidative stress is a dynamic and complex situation characterized by an imbalance between the generation of ROS and the availability and action of antioxidants [4]. The escape of ROS from the antioxidant mechanisms and their progressive accumulation trigger the mechanisms of LPO, as well as the structural damage to proteins and DNA [5].

A state of oxidative stress induces toxic effects of oxidative lipid, protein, and carbohydrate and nucleotide oxidation in the cell, resulting in accumulation of intracellular aggregates, mitochondrial dysfunction, excitotoxicity, and apoptosis. This oxidative damage is common in neurodegenerative diseases and is not clear whether it contributes by initiating the process or is a consequence of it [6,7].

The CNS consumes large amounts of oxygen to carry out the physiological processes, which leads to a high generation of free radicals. Some factors make the CNS susceptible to ROS attack, such as the deficiency of antioxidant mechanisms, the high composition of polyunsaturated fatty acids and the selectivity of the blood-brain barrier, which reduces the diffusion of some antioxidants such as vitamin E [8].

There are also other factors that favor the sensibility of brain to oxidative stress such as high-energy requirement, high levels of iron and lipids and autoxidable catecholamines and low levels of certain endogenous antioxidant molecules [9].

New evidence indicates the presence of oxidative stress in multiple CNS disorders. Recent studies in autistic children showed that imbalance between oxidant / antioxidants species may contribute to the pathogenesis of this disease. Patients have changes in membrane fluidity, neuronal loss in the cerebellum and alteration of the markers of oxidative stress [10].

Glutathione is involved in the neurological protection against oxidative stress and neurological inflammation present in the autistic, improving antioxidative systems. Reduce oxidative stress can be a potentially promising treatment in cases of autism [11].

Antioxidant deficits and impaired methylation ability in children with autism can cause cell damage and epigenetic alterations in gene expression [12].

Taking into account the risk involved in oxidative stress maintained at the cerebral level for patients with some degree of neurodevelopment disturbance, it is very important to try to maintain an adequate redox state, which can be achieved with the use of antioxidant product [13].

Different researchers have used pyridoxine to treat autistic patients, almost all of these studies found that between 30% and 40% of children and adults benefited from a high dose of vitamin B6 supplements with magnesium. Vitamin B6 is required for over a thousand enzymatic reactions, including the production of major neurotransmitters such as serotonin, dopamine, glutathione, hemoglobin and others. The possible explanation is that some children and adults with autism have a diminished ability to convert vitamin B6 to its active form, and defective enzymes to produce key neurotransmitters that require an abnormally high amount of the active form of the vitamin B6 [14].

In one study, 500 mg of vitamin C (ascorbic acid) was found to raise glutathione levels by 50% in college students [15].

It was also found that a treatment with folic acid in children with autism at doses of 800 mcg normalized SAM and partially improved levels of cysteine, total plasma glutathione and the ratio of oxidized glutathione to total glutathione [16].

On the other hand, chronic oxidative stress can lead to mitochondrial dysfunction, increased oxidative stress could be responsible for mitochondrial dysfunction observed in similar cortical regions in the brains of children with ASD. As folic acid is essential for the production of purines and pyrimidines, nucleotide precursors of RNA and DNA, low levels of folate can result in abnormalities in cell proliferation, as well as transcription and translation, and thus contribute to DNA and chromosome instability. It is possible that if earlier in life nutritional supplementation with folate begin, then would be more effective. High doses of folate daily for some months seem to be necessary to achieve the desired changes [17].

The CNS consumes large amounts of oxygen to carry out the physiological processes, which leads to a high generation of free radicals. Some factors make the CNS susceptible to ROS attack, such as the deficiency of antioxidant mechanisms, the high composition of polyunsaturated fatty acids and the selectivity of the blood-brain barrier, which reduces the diffusion of some antioxidants such as vitamin E (α- tocopherol) [8].

Taking these arguments into account, it was decided to carry out the present investigation to evaluate the effectiveness of the application of an antioxidant therapy in patients with primary autism. To achieve this goal, markers of oxidative damage and antioxidant defense capacity will be determined before and after the conclusion of the antioxidant treatment in patients with autism.

MATERIALS AND METHODS

An applied research was carried out, which consisted of a quasi-experimental study of cases with primary autism treated at the Clinical Genetics National Reference Service in Juan Manuel Márquez Pediatric and Mayabeque Neurodevelopment Consultation, Cuba; during October 2014 to June 2016. The universe consisted of 74 autistic children previously evaluated with psychiatrists that are part of the project. Psychiatrists certified the clinical diagnosis of autism and clinical genetics search possible causes of secondary or syndromic autism then were ruled out. In this way selection biases were avoided.

The sample was made up of 40 children who fulfilled the following:

INCLUSION CRITERIA

Individuals of both sexes, from 3 years of age up to 7 years, with diagnosis emitted by psychiatry and clinical genetics of primary autism.

The parent or guardian agrees to participate in the study.

EXCLUSION CRITERIA

- Patients with immunological disorders, chronic diseases, chromosomal or monogenic syndromes.
- Patient who once offered their consent to participate decides not to continue in the investigation or does not complete the studies.
- In order to perform the oxidative stress tests in the cases, it was considered as criteria for taking the blood sample that:
- Children have not had acute infectious diseases in the past month. In positive case, the blood sample was obtained a month later.
- They did not receive radiation (X-rays) a week before the study. In positive case the blood extraction was one month after receiving the radiation.

Oxidative stress studies were performed with 8 hours of fasting. 5 ml of venous blood was obtained by venipuncture and was dispensed in tubes with EDTA. It was transported immediately in thermo-refrigerated at 8°C to the laboratory before 4 hours after the extraction.

Once in the laboratory, the plasma was obtained by centrifugation (2500 rpm for 5 minutes at 4 °C) and the lysate of

erythrocytes by washing with cold 0,9% NaCl solution and lysis with cold distilled water. The concentration of hemoglobin in the lysate was determined and all the samples were stored at -20 ºC until the determination of oxidative stress.

If the oxidative stress was altered, a diet and antioxidant therapy was indicated for a period of 6 months. Test was repeated to evaluate the child's post-treatment REDOX status.

The variables studied and the reference values used in the Oxidative Stress Laboratory of the Medical Genetics National Center with a 95% CI were the following:

MARKERS OF OXIDATIVE DAMAGE

- Oxidative lipid damage (MDA): 0,53 - 0,87 µmol / L
- Oxidative protein damage (AOPP): 26,82 – 50,70 µmol / L
- Concentration of peroxides (FOX): 1,00 - 2,81 µmol / L

ANTIOXIDANT DEFENSE MARKERS

- Enzymatic activity SOD1: 161,83 - 177,35 U / ml
- Enzymatic activity CAT: 67,61 - 78,14 U / ml
- SOD / CAT ratio: 2,23 - 2,64
- Total antioxidant capacity: 0,14 - 0,18 mM Fe "/ L
- Concentration of thiol groups (GSH): 20,06 - 37,44 µmol / L
- Enzymatic activity of glutathione peroxidase (GPx): 29913,46 - 52807,44 mU / ml
- Enzymatic activity glutathione S-transferase: 24,74 - 841,15 U / L

THE INDICATIONS ARE AS FOLLOWS

Diet: Recommendation

- Fresh meat (the chicken without the skin), eggs, vegetable oils, vegetables and fresh fruits with daily consumption is recommended. Legumes, dried fruits, cereals such as rice and corn are recommended too. They can consume frozen food like fruits, vegetables, meats and seafood in natural or in oil. If it is confirmed that the child has dysbiosis due to candida albicans in fecal tools then sugar and honey should be limited. Inlays are not recommended. Also avoid soft drinks, especially with colorants, drinks should be basically natural.

Food not recommended for being rich in gluten and casein

- Milk and its derivatives, wheat: in bread, soups, pizzas, spaghetti, cakes, etc., rye, barley, oats, chocolates with milk, consomme powder (monosodium glutamate), soy, English sauce, Ketchup, vinegar and margarine preferably little butter. Also malt, artificial dyes and flavors, additives such as: BHA, BHT, nitrates, nitrites, caffeine, aspartame, polysorbate 60 and 80, saccharin, quinine.

Antioxidant therapy for day

- Vitamin C - 500 mg, vitamin E – 100 UI, vitamin B6 – 10 mg, folic acid – 1 mg.

Statistical analysis for all objectives used summary measures for qualitative variables (percentages) and quantitative variables (mean and standard deviation).

The values obtained for oxidative damage to the biomolecules, the levels of reactive species and the antioxidant responsiveness of the patients in the two sampling times were compared. According to the distribution of the data and the homogeneity of variance, the relevant statistical tests were applied in each case (t-student test). In all hypothesis tests, a significance level of 0.05 was set.

The program SPSS 15,0 and Microsoft Excel were used. The conclusions of the investigation and the recommendations that were considered pertinent were made.

In the present investigation the ethical principles for medical research established in the Declaration of Helsinki were fulfilled. All the participants were informed about the general characteristics of the study, its objectives, as well as all the information that was required besides the data and samples that had to contribute when deciding to participate. Once verbal consent was obtained from parents or guardians who voluntarily wanted to participate in the study, they were given the informed consent form for their signature.

RESULTS AND DISCUSSION

We studied 40 cases with primary autism and determined the markers of oxidative damage and those of antioxidant defense, before initiating the antioxidant therapy and afterwards, there being a predominance of male patients with 31 cases that represented 77,5% of the total of the sample (Figure 1).

These results coincide with that reported in other researches, where a prevalence of autistic spectrum disorders predominates in males. There are reports of prevalence between 1/42-54 in males and 1/189-252 in females [18,19].

Establishing a male-female relationship, a similar 3:1 (male: female) relationship was found in two other studies, reporting that it is four times more common in boys than girls [13]. Other investigators with similar results have proposed possible evidence of pleiotropic effect and interactions with genes involved in the endocrine system. A "female protective model" has been proposed based on genetic studies. For example, it has been shown that girls' DNA shows resistance to genetic insults in the sense that they are more likely to have extreme genetic mutations related to neurodevelopment, including variants of the number of copies and variants of a single nucleotide that the men, however, they have the same symptoms [19,20].

Several alterations have been identified in the X chromosome of autistic patients, suggesting their participation in the genesis of the prevalence imbalance between males and females. NLGN3, NLGN4X, ARX, MECP2 and FMR1 genes, microdeletions and aneuploidies are include, however , an exome sequencing study has estimated that only 1,7% of men with autism have SNV with loss of function linked to the X chromosome [21].

Table 1 show the results obtained in the determination of markers of oxidative damage and antioxidant defense in patients with autism before and after treatment. As can be seen, the markers that were modified after treatment were the concentrations of MDA, AOPP and FOX. No changes were observed

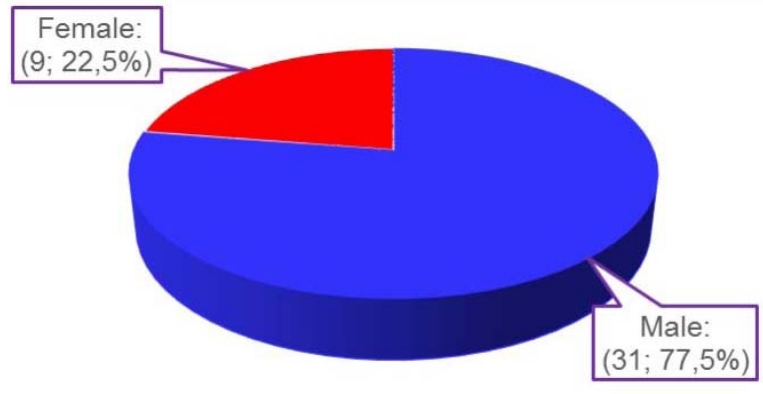

Figure 1 Distribution of patients with primary autism by sex
Source: Compiled information sheet.

in endogenous antioxidant responsiveness, only a tendency to increase total plasma antioxidant capacity was observed without becoming significant.

In a particular way, MDA was initially elevated in 62,5% of the cases and in the control performed at 6 months after treatment the 27,5% of them had normalized results, while 35% maintained high values. The p-value obtained was not statistically significant (0,481).

LOP has been reported to increase in the plasma of children with autism compared to neurotypical children [22]. LOP is a chain reaction between polyunsaturated fatty acids and ROS, and produces lipid peroxides and hydrocarbon polymers that are highly toxic to the cell [23]. MDA is a final product of the peroxidation of polyunsaturated fatty acids and related esters and is therefore used as a LOP marker [24].

Chauhan et al., also described elevated plasma MDA concentrations in 87% of autistic patients [25]. Several publications mention the selectivity in the food intake of autistic children, together with this they report eating rituals and the refusal to eat certain foods. For example, these children eat less vegetables and fruits, which may be related to the results obtained because it is not only about the therapy, but also the compliance with the indicated diet is very important [25,26]. A result similar to that found in this study was reported by two other researchers, in which there is also a considerable increase in MDA [27,28]. Another analysis similar to this one shows that 19% of patients who did not have improvement, about a third of them were not following the diet free of gluten and casein, and still had many peptides of these in their blood [29,30]. This was also possible to verify when interviewing the mothers and relatives of the children in the study sample who considered the difficulty of carrying out the diet with accuracy.

Proteins can be affected by ROS. In relation to AOPP, 65% of the sample studied had high initial values, 47,5% of them in the recontrol normalized the marker, while 17,5% remained elevated, statistical significance was obtained with a McNemar test <0,001.

Results similar to those reported in this research were found in a study in which they stated that AOPPs also improved substantially with antioxidants [31]. Another review refers to the mechanisms of neutralization and defense, which the cell has to remove free radicals and within them include vitamin C and vitamin E along with other endogenous antioxidants [32]. Another of the oxidative damage markers studied were total peroxides, where the highest number of patients in the study were found to have decreased FOX before initiating anti-oxidant therapy (40%) and remained post-treated (30%). In 27,5% of cases, this indicator was normal at onset and after therapy was increased. The p-value obtained was statistically significant (0,001).

It should be noted that although this indicator showed a considerable decrease after therapy, its value remained within the established range, which, according to other authors, is very important if one takes into account that reactive oxygen species play an important role in REDOX signaling and its decrease (below normal) can cause damages mainly of immune system [27-29].

Within the antioxidant enzymes, when evaluating SOD1 no significant changes were observed in the activity of this one after the six months of treatment. It is important to emphasize that 40% of the patients had elevated this marker before the intervention and it was possible to normalize only in 17,5% of the cases after treatment. Statistical analyzes were not significant (Marginal homogeneity test: 0,611 $p \leq 0,05$). On the other hand, the enzyme catalase (CAT) was decreased at the start of the study in 60% of the cases, of which 40% remained post-treatment. Statistical significance was not obtained in this indicator ($p \geq 0,879$).

Antioxidant enzymes such as SOD1 play an important role in cell protection against oxidative damage, which converts superoxide radicals ($O2^{\bullet}$-) into hydrogen peroxide (H2O2) [33]. In relation to SOD1, no significant changes were observed in the activity of this enzyme after six months of treatment. The increase in the activity of this enzyme has been reported for other neurodegenerative diseases, explained as a compensatory mechanism of the organism to prevent tissue damage [31]. In another study, Aksoy et al., also found elevated levels of the activity of the SOD1 and CAT enzymes; they suggest that this increase may be an indicator of the constant presence of oxidative stress [34].

Table 1: Markers of oxidative stress in patients with primary autism before and after the antioxidant treatment.

First	Second sample			p
	↓	N	↑	
MDA				
N	-	8 (20,0 %)	7 (17,5%)	0,481[a]
↑	-	11(27,5%)	14935,0%)	
AOPP				
N	-	13(32,5%)	1(2,5%)	<0,001[a]
↑	-	19(47,5%)	7 (17,5%)	
FOX				
↓	12(30,0%)	3(7,5%)	1(2,5%)	0,001[b]
N	11(27,5%)	2(5,0%)	-	
↑	8 (20,0%)	3(7,5%)	-	
SOD1				
↓	4 (10,0%)	5 (12,5%)	1(2,5%)	0,611[b]
N	6 (15,0%)	4 (10,0%)	4 (10,0%)	
↑	6 (15,0%)	7 (17,5%)	3(7,5%)	
CAT				
↓	16(40,0%)	4 (10,0%)	4 (10,0%)	0,879[b]
N	4 (10,0%)	2(5,0%)	1(2,5%)	
↑	4 (10,0%)	10(25,0%)	6 (15,0%)	
R – SOD1/CAT				
↓	2(5,0%)	3(7,5%)	1(2,5%)	0,070[b]
N	2(5,0%)	3(7,5%)	5 (12,5%)	
↑	5 (12,5%)	10(25,0%)	9 /22,5%)	
Total Antioxidant Capacity				
↓	1(2,5%)	-	4 (10,0%)	0,895[b]
N	-	2(5,0%)	6 (15,0%)	
↑	1(2,5%)	7 (17,5%)	19(47,5%)	
GSH				
↓	10(25,0%)	6 (15,0%)	6 (15,0%)	0,123[b]
N	2(5,0%)	1(2,5%)	2(5,0%)	
↑	6 (15,0%)	5 (12,5%)	2(5,0%)	
GPx				
↓	2(5,0%)	5 (12,5%)	1(2,5%)	0,115[b]
N	6 (15,0%)	6 (15,0%)	10(25,0%)	
↑	2(5,0%)	1(2,5%)	7 (17,5%)	

Legend: ↓ - Diminished, N – Normal, ↑ - Increased, a: Chi cuadrado ($\chi 2$) with correction, b: Fisher Test
Source: Compiled information sheet.

The superoxide dismutase family consists of a group of proteins that use metals such as copper, zinc and manganese for their catalytic activity. The main function of these enzymes is the elimination of superoxide anion, forming the hydrogen peroxide. The results derived from this study suggest the use of trace elements such as Selenium, Manganese and Copper to further improve antioxidant capacity [33]. These are important elements to be taken into account for subsequent studies and are in turn issues that could have a negative influence on similar studies report the decrease in the activity of this enzyme, which can lead to the accumulation of hydrogen peroxide and therefore to the presence of oxidative conditions at the systemic level in these patients [35].

In other studies in which this indicator has been altered, it is argued that catalase works as a compensatory mechanism to maintain an adequate balance of enzymatic systems at the erythrocyte level. From this point of view it can be said that the elevation of SOD1 did not achieve a significant increase in CAT in our patients as reported in other studies. In addition, if we consider that CAT elevation is a compensatory mechanism that increases the efficiency of the antioxidant barrier at the intra-erythrocyte level [36]. the presence of alterations in the level of

oxidative stress in these patients.

The SOD / CAT ratio was elevated in 60% of the sample, and of this only 25% was able to normalize after the treatment. 22,5% of cases maintained this indicator high in both controls.

No statistical significance was found ($p \geq 0,05$).

Some authors suggest a low activity of antioxidant enzymes in autistic children and suggest this finding as a biomarker in autism [37-39]. In the literature it is pointed out that when the SOD / CAT ratio is elevated by low CAT activity, this causes high levels of hydrogen peroxide and hydroxyl radicals ($OH^{\bullet}$) to be generated which cause oxidation of biomolecules [40]. Another article points out that the antioxidant system SOD / CAT acts in the presence of high concentrations of hydrogen peroxide [41]. from this point of view, it would be natural to take into account that total peroxides (FOX) are decreased in the highest percentage of cases. It may be necessary to take into account what McGinnis suggests suggesting the zinc supplementation association, since it is estimated that dietary deficiency causes a total decrease of glutathione, vitamin E, glutathione peroxidase, SOD1 and CAT with the consequent potentiation of oxidative stress and increase of POL and accumulation of free radicals [42].

Extracellular antioxidant defenses are measured through the so-called total antioxidant capacity, in which the cumulative action of all the antioxidants present in plasma and body fluids is considered [40].

Total Plasma Antioxidant Ability did not show statistical significance ($p = 0,895$). The highest number of patients initially had elevated this parameter (67,5%) and remained elevated after treatment in 47,5%; there were no patients with this marker initially decreased and normal thereafter.

Regarding total antioxidant capacity, despite not finding statistical significance ($p = 0,878$), the highest number of patients had elevated total antioxidant capacity and remained elevated after treatment. It is considered that the total antioxidant capacity dampens the generation of hydroxyl radicals ($OH\bullet$); which diffuse into the extracellular fluid [38]. These results are expected if we take into account that initially AOPPs were elevated and that MDA remained elevated in several patients even after treatment.

In the first control, plasma free protein thiol levels (GSH) were decreased in 55% of the cases and 25% of them in the second determination maintained decreased values. No statistical significance was found in this indicator ($p = 0,123$).

The determination of the free protein thiols allows the quantification of the sulfhydryl groups, mainly referred to the free cysteine residues and the glutathione present in a deproteinized sample. This compound is well studied in diseases related to oxidative stress, since it is one of the most sensitive to oxidative disturbances [42,43].

In this indicator, statistical significance was not found due to the use of therapy ($p = 0,981$), observing that 25% of the sample had low GSH levels both before and after treatment, which coincides with a similar study conducted in the United States in which it is argued that in order to obtain the total normalization of glutathione in plasma, vitamin B12 should be associated with antioxidant therapy, this may be something to be considered for later studies [16]. On the other hand, antioxidant therapy facilitated that 15% of the children in the sample normalized their GSH values, which also coincides with the aforementioned study. In other studies reviewed, vitamin B6 is discussed as a treatment for autism. Almost all of these studies found that between 30% and 40% of children and adults benefited from a high dose of vitamin B6 supplements with magnesium, [14]. a higher dose may be required for GSH normalization in the sample studied.

Also in a revised study, the increase in thiol group concentrations suggests the existence of mild oxidative conditions, considering that GSH plays many important metabolic functions, one of which is to protect the cell against free radicals, peroxides and other toxic compounds, as well as protect against the harmful effect of radiation [11]., it is possible to state that according to the reported values of the oxidant indicators a high number of patients in the sample is exposed to stress oxidative.

GPx is one of the enzymes involved in the so-called "redox cycle of glutathione". GPx is a selenium-dependent enzyme, which catalyzes the reduction of hydrogen peroxide and lipoperoxides (L-OOH) and uses reduced glutathione (GSH) as a reducing agent. It is located in: cytosol (erythrocytes), lysosomes (neutrophils, macrophages and other cells of the immune system) [41]. The activity of this enzyme had a tendency to increase, but without becoming significant ($p = 0,083$) after the treatment was applied. This tendency could be related to the observed decrease of peroxide levels, substrate of this enzyme.

Several authors have described that in the autistic, the activity of glutathione peroxidase and other antioxidant enzymes are diminished [11,44]. The results obtained in the present study reaffirm these findings, observing that in general the patients present a low antioxidant response capacity of enzymatic type, which was not modified despite the intervention. Some authors consider the use of antioxidants as controversial considering the clinical experiences in neuroprotection that have been generally negative, fundamentally with the classic antioxidants like vitamin C and vitamin E because the selectivity of the blood brain barrier reduces the diffusion of the latter [8]. However, others suggest the need for concomitant antioxidant treatment with a diet rich in antioxidant vitamins in their natural sources and not in supplements, since this allows them to interact synergistically with other organic and inorganic nutrients present in foods, thus enhancing the antioxidant capacity [45]. The results presented above are attributed to the non-compliance mentioned by the parents in the diet of the patients studied, since the two methods (diet and vitamin treatment) are complemented to achieve modifications in the parameters studied.

Autism is a very complex disorder and involves genetic and non-genetic factors that are not very well understood. To date, several biomedical disorders have been identified and associated with autism, some of which can be treated at least partially. By complying with diet and treatment oriented, many individuals may have clinical improvements, usually slow over months and years. Sometimes a treatment produces great benefits, but the most common is that each treatment helps in a small proportion, because we must not forget as a multifactorial disease its important association with multiple non-genetic factors, in

addition the clinical characteristics of children with autism resistant to food with certain products that constitute important pillars of the diet and in the sample studied was not able to meet the required quality, which masks the results reported in the study.

CONCLUSIONS

In general, results of the first sample showed an increase in the indicators of oxidative damage and values close to normal in the antioxidant defense variables. Subsequent to therapy a high percentage of patients improved the results of advanced protein oxidation products and total peroxide values, but not in the results of MDA in which the highest proportion remained elevated after treatment. As for the antioxidant defense markers, no significant results were found with the administration of the treatment, despite the indication of a dietary pattern, the mothers reported not being able to fulfill it fully given the behavioral characteristics of the children with autism, the reported improvements are attributable to the antioxidant treatment with vitamins. It was concluded that: 1) markers of oxidative damage to proteins and peroxide levels were the best response to the antioxidant treatment used, 2) antioxidant therapy was not able to modify antioxidant response capacity and 3) antioxidant therapy with medication when not supplemented with an adequate diet, is not effective in achieving good regulation of the REDOX status in autistic children.

There are certain limitations that must be taken into account when interpreting the results. First, the sample size is not large enough to generalize the results, and although it provides the statistical power needed for quantitative analyzes, some categorical analyzes may suffer power limitations because of the relatively small number of cases. Secondly, the antioxidant therapy used was limited to 4 vitamins: vitamin E, vitamin C, Vitamin B6 and folic acid, but other authors have described good results with the use of a superior number of vitamins and trace elements that could be taken account for future studies.

Current results may have implications for future research in the identification of biomarkers in patients with neuropsychiatric diseases, especially autism and clinical practice. We suggest performing routine oxidative stress studies in patients diagnosed with autism and applying antioxidant diets and therapies if necessary, making evaluative cuts to identify how the markers studied are modified or not and their impact on neurobehavioral of the patient.

ACKNOWLEDGMENTS

To the Oxidative Stress Laboratory of the Medical Genetics National Center of Cuba for the support offered, to parents and relatives for their participation in the project.

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Cite this article

Hernández DQ, Cruz AL, González SB, Vila DT, Robert YC, et al. (2017) Variation of the REDOX Status in Patients with Primary Autism after Antioxidant Therapy. J Neurol Transl Neurosci 5(2): 1085.