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Antioxidants as a Potential Therapy for Reduction of Oxidative Stress in Autistic Children

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Abstract

Background: Recently, several studies showed oxidative stress in autism spectrum disorder (ASD) patients. Another study found oxidative stress state and mal detoxification of aluminum in autistic children. Zinc, vitamin C and vitamin E may play a significant role in the detoxification and overcoming the oxidative stress among children with autistic disorder.

Objectives: A clinical trial was designed to evaluate the role of vitamin E, vitamin C and zinc oral supplementation on the oxidant-antioxidant status and the aluminum level among autistic children.

Methods: Hair aluminum level (Al), malondialdehyde (MDA), Nitric Oxide (NO) and Glutathione S-Transferase (GST) activity, were estimated in 30 autistic children before and after vitamin E, vitamin C and zinc supplementation for three months.

Results: After supplementation, the results revealed that hair aluminum level, MDA and NO of the children were significantly decreased. While, GST activity was significantly increased. In addition, the study showed improvement in childhood autism rating scale (CARS) score after antioxidant supplementations. As before supplementation, 7.8% of children were scored mild, 51.4% were scored moderate, and 40.8% were scored severe. While, after supplementation there were not any sever cases observed between examined children.

Conclusions: Antioxidant supplementation through vitamin E, vitamin C and zinc apparently improved antioxidant status against oxidative stress among ASD children. This improvement in antioxidant status help in decreasing of aluminum level in autistic children with decreasing oxidative stress biomarkers (MDA, NO) and increasing detoxification enzyme (GST), which in turn leads to an improvement in childhood autism rating scale (CARS) score of autistic children. Thus, antioxidant supplementation may help as a protective supplementation from susceptibility to autism development in children with high aluminum level.

Keywords: autistic children, antioxidant enzymes, aluminum level, oxidative stress, vitamin C, vitamin E, and zinc supplementation.

1. Introduction

Vitamins and minerals are essential nutrients for human health due to their essential functions, as they perform hundreds of roles in the body such as healing wounds, bolstering the immune system, repairing cellular damage and help in sustaining our life. Insufficient intake of nutrients and minerals because of poor food plan is a main contributing factor to many child health problems [1,2]. Children with autism occasionally have restricted, self-limited diets [3-5]. Vitamins and minerals are widely

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recommended via 49% of physicians as a supplementation for children with autism [6].

Some studies have depicted the role of Al in autism spectrum disorder [7-9], due to its poor detoxification. The oxidative stress become worse and it is found to be associated with autism severity that significantly lead to ASD progression [8, 9].

Oxidative stress causes mechanisms that are important in inducing ASD. In addition to the gene-gene interactions and gene-environment interaction which may also potentiate oxidative stress in ASD children [9-11]. Oxidative stress is a situation that takes place due to the imbalance between synthesis of reactive oxygen/nitrogen species (ROS/RNS) and the organism's potential to reduce their deleterious effect via anti-oxidative protection systems. ROS is involved in chemical process of defense or detoxification [35]. There are antioxidant protection mechanisms, which can either be enzymatic (catalase, glutathione peroxidase, superoxide dismutase) or non-enzymatic (vitamin C and E, zinc, reduced glutathione, selenium, riboflavin, carotenoids, etc.), which assist in detoxification of ROS [11,12].

Some studies have shown that Glutathione S-Transferases (GSTs) enzymes have a very essential function in antioxidant protection mechanisms via performing the detoxification of xenobiotics and inactivation of endogenous oxidative stress products [13]. In addition to, our recent study observed that autistic children have reduced glutathione activity, and increased oxidative stress [9]. This open the possibility for novel treatments aiming at planning dietary antioxidant interventions in children with ASD.

Some important exogenous antioxidant supplementation which play a role in protection from ROS include vitamin E, vitamin C and some minerals as zinc [14].

Vitamin C is very popular as antioxidant. It is a water soluble vitamin which protect the body against damage from free radicals [15]. Dolske et al [16] found a reduction in autism severity with treatment by high-dose vitamin C (110 mg/ kg).

In addition, vitamin E appears to be one of the most widely available exogenous antioxidants [17]. Vitamin E refers to lipid soluble compounds including four tocopherols and four tocotrienols, with α-tocopherol being the most biologically available and most well-known form [14]. Tocopherols and tocotrienols act as potent free-radical scavengers, which quench fatty acid peroxyl radicals and yield tocopheroxyl radicals. The resulting tocopheroxyl radicals may be reduced by an appropriate reducing agent such as vitamin C to regenerate vitamin E [18]. There is some evidence for decreased plasma concentration of vitamin E in children of ASD. Consequently, some studies mentioned an pressing need for the medical trial of vitamin E as therapeutic intervention in ASD [11].

Moreover, autistic children are suffering from marginal to marked zinc deficiency and treatment with it was reported to decrease symptoms of hyperactivity [19]. Several studies have demonstrated that zinc deficiency increases the production of ROS [20-22]. Zinc is not functioning as an antioxidant by itself but it can play an important role as a co factor for main enzymes involved in the antioxidant system [23-24].

Autistic children display poor eating habits and inadequate nutritional intake which could explain the low level of many vitamins and essential metals [25]. Simultaneously, Mari Basuset et al. [26] showed ASD children to have low levels of vitamin C, vitamin E and Zinc. Consequently, protection from oxidative stress injury is a target of the current study through therapeutic interventions by supplementation for three months with the non-enzymatic defense mechanism of vitamin E (lipid soluble), vitamin C (water soluble) and using zinc as potentiator for defense mechanisms. The aim of this study is to enhance efforts for diagnosis, intervention, and improvement of antioxidant status which may play a role in decreasing the severity of symptoms among ASD children.

1. Subjects and Methods

1.1. Study participants

In our previous study (Said et al., 2021), hair samples were collected from all autistic children (76) for estimation of aluminum level. Also, random venous blood samples (5 ml) were collected from all autistic children after obtaining written consent for estimation of GST gene polymorphism, GST levels, MDA levels, and NO levels. Only 30 autistic children completed the supplementation for 3 months and were included in the study.

1.2. Supplementations dosage

All children received the supplement, and the dosage was adjusted based on baseline measured body weight. A commercial supplement containing Zinc 10mg/5mL syrup was given as follows: 2.5 mL once daily up to 5 years old children and 5 mL once daily above 5 years old child. Vitamin E 400 mg was given once per day for children above 6 years and day after day for children below 6 years. Vitamin C 100 mg/mL (1 ml equal 20 drops) was given as follows: 10 drops per day for children 2-4 years old and 20 drops for 4 to 8 years old children. All supplements were given orally for 3 months.

2.3. Blood samples

About 1.5-2 cm hair samples were collected before and after supplementation by single cutting from the occipital region of each children who completed the study. The Al levels in hair samples were determination using the inductively coupled plasma mass spectrometry (ICP-MS) device. Also, 5 mL blood samples were collected and were divided into two portions; one portion was left to clot for 30 min at 37 °C and then centrifuged at 3000 rpm for 10 min to isolate the serum. The sera were kept at −20 °C for laboratory investigations. The remainder was collected on EDTA for separating plasma for determination of GST enzyme activity.

2.4. Childhood Autism Rating Scale (CARS) determination

Subdivision and detection of severity of autistic children were done before and after supplementation by using the CARS. The CARS consists of 14 domains assessing behaviors associated with autism, with a 15th domain rating general impressions of autism. Each domain is scored on a scale ranging from one to four; higher scores are associated with a higher level of impairment. Total scores can range from a low of 15 to a high of 60; scores below 30 indicate that the individual is in the non-autistic range, scores between 30 and 34 indicate mild, scores between 34.5 and 36.5 indicate moderate autism, and scores from 37 to 60 indicate severe autism [27].

2.5. Estimation of aluminum concentration

Aluminum level in human hair samples were estimated using ICP-MS device. Approximately 100 strands of hair (50 mg) were used. Adhesive paper was placed over the end of the hair strands closest to the scalp; the paper was marked with an arrow indicating the end of hair. The samples were placed in sealed plastic bag. Hair samples were digested in closed vessels in microwave oven using mixture of suprapur®concentrated nitric acid. The digested solution was diluted with deionized water. Certified reference stock standard solutions of aluminum (1000 mg/L) were used.

2.6. Estimation of Glutathione s-Transferase (GST) enzyme activity:

GST was determined by colorimetric method according to Habig et al [28].

2.7. Estimation of serum malondialdehyde (MDA) concentration:

Malondialdehyde level in serum was measured at532 nm using Shimadzu U.V-Visible recorder spectrophotometer model U.V-160 according to Yagi [29].

2.8. Estimation of serum Nitric Oxide (NO) concentration:

Nitric oxide was determined according to Miranda et al [30].

Statistical analysis

The collected data and the laboratory results were computerized. Statistical analysis was carried out using SPSS. The results are expressed as means ± SD. The paired t-test was used to detect changes in the quantitative data after antioxidant supplementation for three months. Using the Wilcoxon signed rank test, the percentage of children with changes in the oxidative-antioxidant parameters and aluminum levels after supplementation compared

to before supplementation was calculated. Significance was considered at p-value ≤0.05.

3. Results

The present study was performed to study the ameliorating effects of the supplement consumption on oxidative state of autistic children and the severity of symptoms scored by CARS. The study was performed on 30 autistic children including 21 male and 9 female with age mean (6.416±1.569) years.

Table (1) showed that aluminum concentration in hair showed a significant decrease (p<0.001) after antioxidant supplementation when compared to before supplementation. Also, the enzymatic activity of GST was significantly increased (p<0.037) after antioxidant supplementation when compared to before supplementation. On the other hand, the oxidative stress markers (MDA, NO) were significantly decreased after antioxidant supplementation (p<0.001, p<0.048) respectively when compared to before supplementation. Also, CARS score was significantly decreased after antioxidants supplementation (p<0.001).

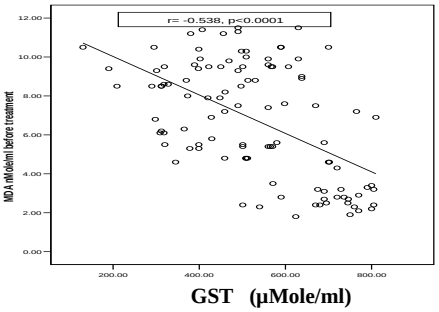
Table 1. The levels of aluminum, the oxidative stress markers, anti-oxidant status and CARS score among autistic children before and after antioxidants supplementation

Parameters	Autistic Children		
	before supplementation	after supplementation	p-value
Al mg/kg	33.1280± 11.02	14.5465±2.8	0.001
GST µMole/ml	476.4667±152.04	569.8333±148.6	0.037
MDA nMole/ml	8.1733±2.05	5.3200±1.38	0.001
NO µMole/ml	2.4133±0.6	1.6500±0.45	0.048
CARS score	36.671±1.88	33.8355±1.45	0.001

Al: aluminum, NO: nitric oxide, MDA: Malondialdehyde, GST: glutathione-s-transferase enzyme, CARS scale: Childhood Autism Rating Scale

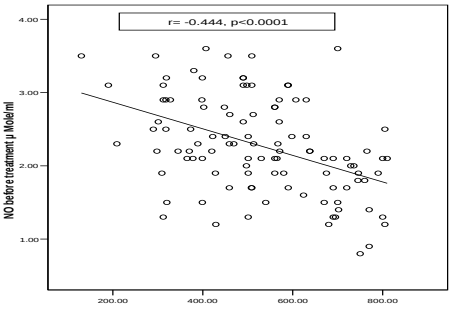
Significant difference at P<0.05

The current study found that there was a negative correlation between oxidative stress markers (MDA& NO) and anti-oxidant status (GST activity) (Figure 1a&b). In addition, The study showed a significant positive correlation between aluminum hair levels and the severity of CARS score among autistic children (Figure1,2 & 3).



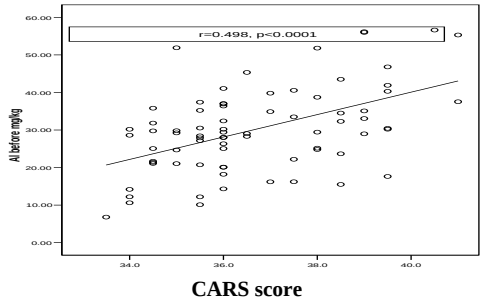
MDA: Malondialdehyde, GST: Glutathione-S-Transferase enzyme

Fig. 1. Scatter plot correlation between malonaldhyde (MDA) and anti-oxidant status (GST)



NO: nitric oxide, GST: Glutathione-S-Transferase enzyme

Fig. 2. Scatter plot correlation between Nitric oxide (NO) and anti-oxidant status (GST)



Al: aluminum, CARS scale: Childhood Autism Rating Scale

Fig. 3. The correlation between aluminum hair levels and the severity of CARS score among autistic children

When CARS score was compared for the children before and after antioxidant supplementations, it showed an improvement in CARS scale after antioxidant supplementations. As before supplementation, 7.8% of children were scored mild, 51.4% were scored moderate, and 40.8% were scored severe. While, after supplementation there were not any sever cases observed between examined children

and the percentage of children with mild CARS score was increased to 55.3% (Figure 4).

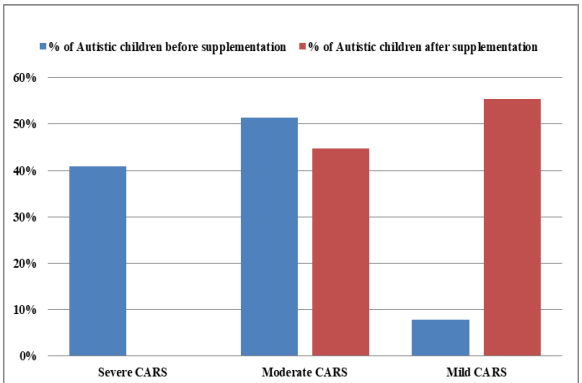


Figure (4) Precentages of CARS scales among autistic children before and after supplementation.

CARS scale: Childhood Autism Rating Scale, Mild autism (30-34), Moderate autism (34.5-36.5), Severe autism (≥37)

4. Discussion

Aluminum is the third most common abundant metal on earth. It is a soluble metal that have changed the world but more exposure to Al has a bad effects on environment and health. Exposure to Al may be a significant contributing factor to cause ASD. A remarkable higher Al concentration was reported in hair of ASD children compared to neuron-typical children [8,9 , 31].

Oxidative stress markers including lipid peroxidation have been observed elevated in ASD children. In addition to antioxidant factors level as vitamin E and vitamin C, and glutathione were decreased compared to the controls [32].

Moreover, one of the most important mechanisms by which heavy metals can result in oxidative stress via overproduction of free radicals or alteration of antioxidant protection mechanisms. Therefore, alteration in oxidative stress can be confirmed by the direct measurement of malondialdehyde (MDA) [33], Literature data showed evidence for increased levels of oxidative stress markers in individuals with ASD [11, 34]. Our previous study showed an increased MDA and NO levels in autistic children [9], and it was recommended to look at the protection role of an antioxidant supplementation for autistic children.

On their relation to antioxidant protection mechanisms, some vitamins act as scavengers for superoxide anion [35]. Several studies recorded high MDA levels in the blood, urine of autistic children. The Egyptian study of Abdel Salam et al. [36] demonstrated elevation in MDA level by 87.3% in autistic children compared to their matched control. Also the study of Maguid et al, [37] noted significant increase in MDA levels among ASD children. Our results showed that, supplementation with vitamin E, vitamin C and zinc was found to be effective for treatment of oxidative stress, as shown by decreasing in the levels of oxidative biomarkers (MDA and NO) after supplementation.

Supplementation with vitamins C and E reduced vascular O2− and improved total plasma antioxidant status [38]. Mechanisms by which antioxidant vitamins E& C affect oxidative stress might be via scavenging of free radical. However, these vitamins may also affect the vascular redox state through synergistic interplay among vitamins C and E [39].

Vitamin E refers to lipid soluble compounds which includes tocopherols and tocotrienols. Tocopherols and tocotrienols act as powerful scavenger for free-radical [40], they quench fatty acid peroxyl radicals and yield tocopheroxyl radicals, the tocopheroxyl radicals may be reduced by vitamin C to regenerate vitamin E. Thus vitamin C acts as a primary substrate for detoxification of ROS, and neutralize it by acting as a secondary antioxidant during reductive recycling of the oxidized form of α-tocopherol (vitamin E) [41,42]. Vitamins C and E have additionally been proven to stimulate activity of nitric oxides (NOS) through increasing intracellular tetrahydrobiopterin, which increases NO synthesis [43,44]. In addition, Zn exerts an antioxidant activities by activating the synthesis of antioxidant enzymes [45].

We conducted this trial based on our previous findings showing an elevation of hair Al level as a result of defect in GST enzyme activity and alterations of oxidative stress biomarkers (MDA& NO) among autistic children [9]. Previous studies have suggested that exogenous antioxidant supplementation can also play an important role in stopping oxidative stress in ASD [46, 32]. The present findings showed that three months vitamin C, vitamin E and zinc supplementation program can significantly decrease Al level in hair of autistic children after supplementation and improve ASD severity. These are consistent with the results of El-Ansary et al [47] who showed that supplementation with vitamin C caused significant reduction in autism severity and improvements in sensorimotor behaviours.

In addition, Frustaci et al [48] reviewed studies about vitamin E and ASD and reported lower vitamin E levels in children with ASD. We suggest that, the improvements seen in hair aluminum level with antioxidants and mineral supplementation in the current study was attributed to improvement in GST enzyme activity. As many studies reported that GST plays a crucial role in detoxification of heavy metals as aluminum [49, 50].

Several findings have related the improvement of enzymes involved in antioxidant defenses to the action of Zn^{2+} [51,52]. As investigated, zinc has

catalytic and a regulatory role and the activity of GST (a key enzyme in detoxification reaction) was significantly lowered in zinc deficiency [53]. Some studies recorded that infants who have autistic disorders, are suffering from marginal to marked zinc deficiency [11, 19] and treatment with it was reported to decrease symptoms of hyperactivity and impaired socialization in ASD patients [53]. Therefore, the researchers attributed the improved glutathione level following Zn treatment to its catalytic and regulatory role.

Our results showed also significant reduction in oxidative stress biomarkers (MDA and NO) among ASD children after antioxidant supplementation. This could be attributed to the improvement in the antioxidant status. As ensured by a significant negative correlation between oxidative biomarkers and GST after antioxidant supplementation in this study (Figure 1).

Overall, spatial arrangement of vitamins C and E facilitates the efficient removal of free radicals, and combinations of vitamins and minerals may be of benefit in amelioration of oxidative stress among autistic children due to the possible synergistic interaction between vitamins C and E to improve oxidative stress and catalytic and a regulatory role of Zn to antioxidant enzyme.

The significant reduction in CARS score and Al level in the present study after supplementation could be attributed to decreasing in oxidative stress biomarkers level (MDA& NO) and improvement of antioxidant enzyme activity (GST). Ozonoff et al [54] supposed the utilization of CARS as a diagnostic approach that classified individuals according to scale ranging from normal to severe and yields a complex tool of diagnosis from non-autistic to mildly, moderately, or severely autistic. In addition, vitamin C is one of the most effective biological antioxidants that can reduce the risk of diseases associated with oxidative stress [55]. A previous study reported that the effectiveness of vitamin C supplementation on reduction of symptoms severity among ASD children [16, 32].

Blaylock and Strunecka (2009) reported that Al causes oxidative stress inside brain tissue, exacerbating the medical presentation of autism via worsening of excitotoxicity. They recommended that the heterogeneous signs of ASD have been connected to the dysregulation of glutamatergic neurotransmission in the brain together with enhancement of excitatory receptor function via proinflammatory immune cytokines as the underlying pathophysiological technique. The present study showed additionally that the hair Al levels were directly proportional to the measured CARS. This was consistent with Blaurock-Busch et al [56] and Elsheshtawy et al [57] who reported an elevation hair concentration of heavy metals among autistic cases, and this elevation was correlated with the severity of symptoms. Also, Adams et al [58]. found that the body level of toxic metals was significantly related to the severity of autism. This directed the attention to the use of nutritional antioxidants that reduce excitotoxicity and brain inflammation as a manner to relieve neurotoxic effects of aluminum [59].

The current study revealed also that, CARS score was improved after antioxidant supplementations. As before supplementation, 7.8% of children were scored mild, 51.4% were scored moderate, and 40.8% were scored severe. While after supplementation, there were no severe cases, 44.3% of children had moderate CARS score and 55.7% had mild score. We attributed this improvement in CARS score to the decrease in oxidative stress state after better detoxification of aluminum and decrease in its level. Genetically, autistic children may be less able to detoxify toxic environmental agents, making them to be suffering from neural damage consistent with autistic behavior [60]. In this regard, antioxidant supplementation to improve aluminum detoxification enzymes including GST, can improve the worsening clinical presentation.

5. Conclusion:

In conclusion, the antioxidant supplementation through vitamin E, vitamin C and zinc improved antioxidant status among ASD children. The improved antioxidant status help in detoxification of Al in autistic children, resulting in decrease oxidative stress biomarkers (MDA, NO) and increase in the detoxification power of GST enzyme. Accordingly, this lead to an improvement in CARS score among autistic children. Hence, antioxidant supplementation may play a protective role against the severity of autism in children with high un-detoxified Al level.

As, the current study was the first study that use combination of vitamin E, vitamin C and zinc as antioxidant supplementation in autistic children, we recommended several studies on a larger sample size.

Conflicts of interest

There are no conflicts to declare

Ethical approval

Ethical approval was obtained from the "Ethical Committee" of National Research Centre.

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