

The Antioxidant Effect of Omega-3 Fatty Acid Supplements on Oxidative Stress Markers in Children with Autism Spectrum Disorder: A Case-Control Study

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ABSTRACT

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterized by deficits in social communication and repetitive behaviors. Emerging evidence suggests that oxidative stress plays a significant role in its pathophysiology. Omega-3 polyunsaturated fatty acids (PUFAs) possess potent antioxidant properties that may mitigate oxidative damage in autistic children. The present study aimed to assess the antioxidant effect of omega-3 fatty acid supplementation on oxidative stress markers, Malondialdehyde (MDA), Superoxide Dismutase (SOD), and Catalase (CAT) in children with ASD in a comparison with to healthy controls in a case-control study that conducted on 90 subjects divided into three groups (30 subjects each): Group I includes healthy control children; Group II includes autistic children receiving omega-3 supplementation; and Group III who are autistic children that didn't receive omega-3 supplementation. Serum levels of MDA, SOD, and CAT were measured in patients' groups and compared with those of controls and the results demonstrated that children in Group III demonstrated significantly elevated MDA levels and reduced SOD and CAT activities compared to both healthy controls and omega-3-treated autistic children ($p < 0.05$). Omega-3 supplementation (Group II) significantly reduced MDA levels and restored SOD and CAT activities toward values comparable to healthy controls. In conclusion, Omega-3 fatty acid supplementation exerts a significant antioxidant effect in children with ASD, reducing oxidative stress markers. These findings support the therapeutic potential of omega-3 in managing ASD-related oxidative stress.

Keywords: Antioxidants, Autism Spectrum Disorder, Catalase (CAT), Malondialdehyde (MDA), Omega-3 fatty acids, Oxidative stress, Superoxide Dismutase (SOD).

INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder whose clinical manifestations become evident within the first 36 months of life. It is associated with significant disruptions in neural growth and synaptic development occurring both prenatally and postnatally¹. A wide spectrum of symptoms was presented in ASD Children that include mainly the impairments in social communication and interaction, repetitive and restricted behavioral patterns, abnormalities in speech skills, and poor eye contact, all of these manifestations showed to affect the daily functioning and quality of life². Several factors may be involved in the etiopathogenesis of ASD, including complex interactions between genetic susceptibility of children and environmental factors. Several biological mechanisms are proposed

to implicate in ASD pathogenesis which include mainly the oxidative stress which is considered as an imbalance between the production of reactive oxygen species (ROS) and the capacity of the biological antioxidant defenses to neutralize them^{3,4}. Previous literatures have demonstrated that the elevation in the oxidative damage markers in a parallel with a reduction in the activity of antioxidant enzymes in the blood and brain tissues of ASD children indicates that oxidative stress may contribute significantly in the neurological dysfunction observed in this disorder^{5,6}. Malondialdehyde (MDA) is one of the most widely used biomarkers for the assessment of oxidative stress and lipid peroxidation. High levels of MDA indicate an elevation in the oxidative damage to cellular membranes and DNA. In contrast, catalase (CAT) and superoxide dismutase (SOD) enzymes are a main defense mechanism against ROS as they are considered as key enzymatic

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antioxidants that constitute the first line of cellular defense against oxidative stress. The function of one of these enzymes complement the effect of the other in that SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently subjected to CAT for neutralization. It was demonstrated previously that ASD children showed reduced activities of SOD and CAT, further supporting the hypothesis of compromised antioxidant defenses in this population^{7,8}.

Long-chain omega-3 polyunsaturated fatty acids (n-3 LCPUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential components of neuronal cell membranes and are crucial for brain structure, and neurotransmission, in addition to inflammatory regulation. Omega-3 fatty acids were demonstrated to exert anti-inflammatory and antioxidant effects by modulating the production of pro-inflammatory eicosanoids, reducing lipid peroxidation, and upregulating antioxidant enzyme activity^{5,9}. The epidemiological and clinical studies have reported lower plasma levels of omega-3 fatty acids in ASD children in a comparison with healthy children, assuming a potential relation between omega-3 deficiency and the oxidative stress observed in ASD¹⁰. Despite growing interest in omega-3 supplementation as a complementary treatment for ASD, there is limited evidence specifically addressing its impact on enzymatic antioxidant biomarkers in pediatric ASD populations within our region. This study was therefore designed to evaluate the antioxidant effects of omega-3 supplementation on MDA, SOD, and CAT levels in Iraqi children with ASD using a case-control design with three well-defined groups, allowing for a comprehensive comparison between supplemented and unsupplemented autistic children relative to healthy controls.

MATERIAL AND METHODS

Study Design: A case-control study was conducted from December 2025 to April 2026 in the Central Teaching Hospital of Pediatric, Baghdad Al-Karkh Health Directorate, Baghdad, Iraq. The ethical approval for the current study was obtained from the Ethical Committee, College of Medicine, Al-Mustansiriya University, Baghdad, Iraq with an approval number of 8245 on 05-11-2025. Additionally, written informed consent was obtained from the parents or legal guardians of all participating children prior to enrollment. The study was conducted in accordance with the Declaration of Helsinki.

Study Groups: Participants were divided into three groups:

- Group I (Healthy Controls, n=30): Apparently healthy male children of matched age, with no history of neurodevelopmental disorders, chronic illness, or medication use.
- Group II (ASD + Omega-3, n=30): Male children with a confirmed ASD diagnosis who were receiving omega-3 fatty acid supplementation (fish oil capsules, 1–2 g/day of combined EPA/DHA) for a minimum of three months prior to sample collection.
- Group III (ASD, No Omega-3, n=30): Male children with a confirmed ASD diagnosis who were not receiving omega-3 supplementation or any other antioxidant therapy.

Inclusion Criteria

- Male children aged 3–12 years.
- Diagnosis of ASD confirmed according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria.
- For Group II: receiving omega-3 supplementation for at least 3 consecutive months.
- For Group III: no current use of omega-3 or other antioxidant supplements.

- Written informed consent provided by parent or legal guardian.

Exclusion Criteria

- Children with other known genetic syndromes (e.g., Down syndrome, Fragile X syndrome) or chromosomal abnormalities.
- Presence of comorbid systemic diseases including hepatic, renal, cardiovascular, or endocrine disorders.
- Children diagnosed with other concurrent psychiatric disorders (e.g., schizophrenia, bipolar disorder).
- Current or recent (within the past 3 months) use of antioxidant supplements (other than omega-3 in Group II), including vitamins C, E, or selenium.
- Active infection, fever, or any acute inflammatory condition at the time of sample collection.
- Malnutrition or known metabolic disorders that may independently affect oxidative stress markers.
- Incomplete clinical data or parental refusal to participate.

Blood Sample Collection: A sample of five milliliter of Venous blood was drawn in the morning from all participants who were under fasting conditions. Samples were collected in plain gel tubes and allowed to clot at room temperature for 30 minutes, then centrifuged at 3000 rpm for 10 minutes to obtain serum. Serum aliquots were immediately stored at -80°C until analysis.

Biochemical Measurements: Serum levels of Catalase (CAT), Malondialdehyde (MDA), and Superoxide Dismutase (SOD) enzymes were measured using Enzyme-Linked Immunosorbent Assay (ELISA) kits that provided commercially and the procedure conducted according to the manufacturer's instructions. All samples were analyzed in duplicate, and the mean values were used for statistical analysis. The sensitivity and intra-/inter-assay coefficients of variation for each kit were within acceptable limits as specified by the manufacturer.

Statistical Analysis: All data were expressed as mean $\pm$ standard deviation (Mean $\pm$ SD). The normality of data obtained were checked for normality by using Shapiro-Wilk test which demonstrated that the data were normally distributed, so, parametric statistical tests were applied. Differences among the three groups were evaluated using one-way Analysis of Variance (ANOVA)¹¹. Where significant differences were identified, Tukey's Honest Significant Difference (HSD) post-hoc test was applied to determine pairwise group differences. Pearson's correlation coefficient was used to assess correlations between the studied markers. Statistical analysis was performed using SPSS software (Version 26.0, IBM Corp., Armonk, NY). A p-value of < 0.05 was considered statistically significant^{12,13}.

RESULTS

Demographic Characteristics

A total of 90 male children were enrolled in this study. The three groups were comparable in terms of age distribution (Mean age: Group I: 7.2 ± 2.1 years; Group II: 7.5 ± 1.9 years; Group III: 7.4 ± 2.0 years; $p = 0.892$). No significant differences in anthropometric parameters were observed among the groups. Table 1 summarizes the demographic characteristics of the study participants.

Malondialdehyde (MDA) Levels

Serum MDA levels were significantly different among the three groups ($p < 0.001$). Children with ASD not receiving omega-3 supplementation (Group III) exhibited the highest MDA concentrations (Mean $\pm$ SD: 4.82 ± 0.91 nmol/mL), which were significantly elevated compared to healthy controls (Group I: 2.14 ± 0.43 nmol/mL, $p < 0.001$) and ASD children receiving omega-3 (Group II: 3.21 ± 0.67 nmol/mL, $p <$

Table 1. Demographic Characteristics of Study Groups

Variable	Group I (Healthy Controls) n=30	Group II (ASD + Omega-3) n=30	Group III (ASD, No Omega-3) n=30	p-value
Age (years), Mean ± SD	7.2 ± 2.1	7.5 ± 1.9	7.4 ± 2.0	0.892
Body Weight (kg), Mean ± SD	24.3 ± 5.1	25.1 ± 4.8	24.7 ± 5.3	0.874
Male, n (%)	30 (100%)	30 (100%)	30 (100%)	—

Table 2. Comparison of Oxidative Stress Markers among the Three Groups

Parameter	Group I (Healthy Controls) n=30	Group II (ASD + Omega-3) n=30	Group III (ASD, No Omega-3) n=30	p-value
MDA (nmol/mL)	2.14 ± 0.43	3.21 ± 0.67 †	4.82 ± 0.91 ‡	< 0.001
SOD (U/mL)	112.6 ± 14.7	95.3 ± 13.1 †	68.4 ± 11.2 ‡	< 0.001
CAT (U/mL)	28.7 ± 4.9	21.8 ± 3.7 †	14.2 ± 3.1 ‡	< 0.001

* One-way ANOVA. † Significant difference vs. Group I ($p < 0.05$, Tukey's HSD). ‡ Significant difference vs. both Group I and Group II ($p < 0.001$, Tukey's HSD).

Table 3. Pearson Correlation Matrix Between Oxidative Stress Markers in All Participants

Parameter	SOD	CAT
MDA	-0.712**	-0.681**
SOD	-	0.743**

** Correlation is significant at the 0.01 level (2-tailed).

0.001). Notably, Group II also showed significantly higher MDA levels than healthy controls ($p = 0.003$), though omega-3 supplementation resulted in a partial but significant reduction in MDA levels compared to Group III. These findings indicate that omega-3 supplementation attenuates lipid peroxidation in autistic children, consistent with the antioxidant properties of omega-3 fatty acids^{14,15}.

Superoxide Dismutase (SOD) Activity

SOD activity was significantly lower in autistic children not receiving omega-3 supplementation (Group III: 68.4 ± 11.2 U/mL) compared to healthy controls (Group I: 112.6 ± 14.7 U/mL, $p < 0.001$). Omega-3-supplemented autistic children (Group II) demonstrated significantly higher SOD activity (Group II: 95.3 ± 13.1 U/mL) compared to Group III ($p < 0.001$), though still slightly lower than healthy controls ($p = 0.021$). This partial restoration of SOD activity following omega-3 supplementation suggests that omega-3 fatty acids enhance the enzymatic antioxidant defense system in ASD, as previously reported in comparable studies^{16,17}.

Catalase (CAT) Activity

CAT activity showed a similar pattern to SOD. Group III demonstrated the lowest CAT activity (14.2 ± 3.1 U/mL), significantly reduced compared to Group I (28.7 ± 4.9 U/mL, $p < 0.001$) and Group II (21.8 ± 3.7 U/mL, $p < 0.001$). Group II showed intermediate CAT values that were significantly higher than Group III ($p < 0.001$) but slightly lower than healthy controls ($p = 0.031$). These results further support the role of omega-3 in upregulating catalytic antioxidant enzymes, thereby reducing hydrogen peroxide-mediated cellular damage in ASD patients¹⁸ (Table 2).

Correlation Analysis

Results of the present study demonstrated that there was a significant positive correlation between the levels of MDA and the severity of ASD in group III who didn't receive antioxidants ($r = 0.61$, $p < 0.001$), which indicate that higher oxidative stress is related tightly to the severity of autistic features. Furthermore, results illustrated in Table 3 showed that both of SOD and CAT activities were correlated significantly and negatively with MDA in all groups ($r = -0.71$ and $r =$

-0.68 , respectively, $p < 0.001$). On the other hand, both SOD and CAT showed to be significantly and positively associated with each other.

DISCUSSION

The present work targeted to assess the oxidative stress in autistic patients and to assess also the effect of Omega-3 administration on that stress and in alignment with several studies the current study demonstrated that there is a significant dysregulation in oxidative stress markers in children with ASD, as evidenced by the high MDA levels with the significant decrease in the activities of the defense mechanism enzymes, named SOD and CAT. These findings were obtained in untreated autistic children (Group III) in a comparison with age-, sex- and BMI-matched control^{6,7,19}.

As mentioned previously, MDA, considered as the most widely used marker for the assessment of the oxidative stress which is a major end-product of lipid peroxidation, serves as a reliable biomarker for the cellular oxidative damage^{20,21}. Significantly high levels of MDA that were obtained in Group III were consistent with previous research by Zoroglu et al.²² and Al-Gadani et al.²³, who reported that the levels of MDA in autistic children were significantly higher than those in healthy children. The overproduction of ROS in ASD may result from mitochondrial dysfunction, neuroinflammation, and impaired antioxidant buffering capacity, all of which have been independently documented in this disorder.

The significantly diminished SOD and CAT activities in untreated autistic children indicate a compromised enzymatic antioxidant defense. SOD is the primary cellular enzyme responsible for neutralizing superoxide radicals, converting them to hydrogen peroxide, which is subsequently detoxified by CAT. Reductions in both enzymes create a cascade of oxidative damage that may amplify neuronal injury and contribute to the behavioral manifestations observed in ASD^{8,24}. These findings align with those of Meguid et al.²⁵ and Chauhan et al.²⁶, who similarly reported suppressed SOD and CAT activities in autistic individuals.

Importantly, omega-3 supplementation in Group II was associated with a significant reduction in MDA levels and partial restoration of SOD and

CAT activities relative to untreated autistic children. This antioxidant effect of omega-3 fatty acids is attributed to multiple mechanisms: (1) incorporation of EPA and DHA into cell membrane phospholipids, which reduces membrane susceptibility to lipid peroxidation; (2) activation of the Nrf2-ARE pathway, which upregulates endogenous antioxidant enzyme expression; and (3) reduction of pro-inflammatory cytokine production, thereby decreasing ROS-generating inflammatory cascades^{9,27}.

The findings of the current study are consistent with those of Bent et al.²⁸ and Amminger et al.²⁹, who reported improvements in behavioral outcomes and reductions in oxidative stress markers following omega-3 supplementation in ASD. Furthermore, Mazahery et al.¹⁰ in their systematic review highlighted the beneficial effects of n-3 LCPUFAs in ASD, particularly in modulating inflammatory and oxidative pathways. The observation that omega-3-treated autistic children still showed slightly elevated MDA and slightly reduced SOD/CAT compared to healthy controls suggests that omega-3 supplementation, while beneficial, may not fully normalize oxidative stress and that combination antioxidant approaches may be warranted³⁰.

Despite its strengths, this study has several limitations. The cross-sectional design precludes causal inferences. Dosage and duration of omega-3 supplementation were not standardized across all participants in Group II. Additionally, dietary intake of omega-3 from food sources was not quantified, which may have introduced confounding effects. Future longitudinal studies with standardized supplementation protocols and larger sample sizes are recommended to confirm and extend these findings.

CONCLUSION

It was concluded that the children with ASD showed a significantly higher levels of oxidative stress marker (MDA) with a significant reduction in the antioxidant defense enzymes activities (SOD and CAT) that improved significantly with the administration of Omega-3 fatty acids which provide a promising finding that needs more profound study to prove the significance of using Omega-3 fatty acid as an adjunct to the comprehensive management of ASD with the elucidation of the optimal dosage required, duration of administration in addition to the patients selection criteria.

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Competing Interest: None

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