ORIGINAL ARTICLE

Brain derived neurotrophic factor, oxidative stress status and vitamin D levels in patients with autism spectrum disorder

Moushira Zaki, Hala T. El-Bassyouni, Khaled Hamed, Rehab Selim Ismail Moustafa and Eman R. Youness

ABSTRACT

Background: Brain Derived Neurotrophic Factor (BDNF) may play a role in the progression and development of the autism spectrum disorder (ASD). The aim of the study was to evaluate oxidative stress marker malondialdehyde (MDA), vitamin D status (serum 25-hydroxy vitamin D) and brain-derived neurotrophic factor (BDNF) in children with autism and elucidate whether there is a correlation between the severity of the disease and the level of the BDNF.

Patients and methods: 94 children with ASD (75 males and 19 females) and 72 healthy controls matched in age were enrolled. The oxidative stress markers were measured by evaluating serum levels of MDA, 25 - hydroxy vitamin D in patients and controls and BDNF concentrations were measured.

Results: Vitamin D (25(OH)-D) was statistically lower in the patients compared to controls (P <0.006). The MDA and BDNF serum levels were statistically higher in children with ASD (P < 0.001) related to the control group. The BDNF levels correlated with the severity of the disease.

Conclusion: The levels of BDNF could be considered a diagnostic or prognostic indicator of ASD, it is recommended to evaluate its role in the onset and progression of this disorder. Our study sheds light on the importance of supplementation of ASD patients with antioxidants and Vitamin D as it may help in the amelioration of their symptoms.

Keywords: Autism spectrum disorder (ASD), brain-derived neurotrophic factor (BDNF), malondialdehyde, oxidative stress, Vitamin D (25(OH)-D)

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INTRODUCTION

There is increasing prevalence of ASD in children all over the world and scarcity of therapeutic remedies hence, ASD became one of the most challenging disorders to both diagnose and treat. (1). Autism is considered as a spectrum with a variable presentation that ranges from mild to severe and includes the presence of repetitive and abnormal motor sensory activities. (2). Numerous studies have proposed that various genes may be responsible for the appearance of the cognitive abnormalities and behavioural that characterize ASD (3,4); including both epigenetic and genetic and environmental factors may contribute to the appearance of its clinical phenotype (5).
Children with ASD diagnosis are considered more vulnerable to oxidative stress that plays a role in the clinical manifestations and development (2,6). Early treatment of the oxidative stress may result in a better prognosis as it could prevent irreversible brain damage (7).

Several researchers have identified that a deficiency of vitamin D during pregnancy and in children increases the risk of ASD suggesting the role of vitamin D as an environmental risk factor (8,9). Previous research has indicated that the synaptic dysfunction might be a possible mechanism for the occurrence and progression of the characteristics of ASD (10). Moreover, dysregulation of proteins associated with synaptic plasticity often show in patients with ASD, particularly brain-derived neurotrophic factor (BDNF) (11). The involvement of BDNF in ASD has been confirmed by BDNF protein concentrations and altered BDNF mRNA expression in the blood of patients with ASD (12). BDNF is found in practically every section of the brain and is involved in a variety of neurophysiological processes. (13). The most critical functions of BDNF include the regulation of glycogenesis, synaptogenesis, and neurogenesis as well as neuroprotection and control of long and short duration synaptic interactions that influence memory mechanisms and cognition (14). While there is a strong link between BDNF levels in the blood and the central nervous system (CNS) in rats, proof of this link in people is still missing. Nevertheless, it is expected that peripheral levels of BDNF indirectly reflect the levels of BDNF in the brain. Subsequently, the BDNF concentration in peripheral blood could be considered a potential biological marker in evaluating ASD individuals (10,13–16).

PATIENTS AND METHODS

In this study, 162 children (94 ASD and 72 healthy controls) were recruited from the Clinical Genetics Department, National Research Centre, Egypt. The diagnosis was established according to the parameters founded in the Diagnostic and Statistical Manual of Mental Disorders DSM-5 (17). The severity of ASD was determined using the Childhood Autism Rating Scale (CARS) (18). CARS scores range from 15 to 60, and the cut-off point for an autism diagnosis is a score of 30 or above. According to the scoring standards of CARS, scores between 30 and 37 indicate mild to moderate autism and scores between 38 and 60 are characterized as severe autism, 64.9% (61/94) of the children in the study group had a severe degree of ASD. Gastrointestinal tract (GIT) disturbances were recorded during clinical examination.

The human BDNF was measured by immunoassay ELISA kits, manufactured in Germany (Catalogue No. DBDOO, R & D systems) according to manufacturer's instructions. To assess vitamin D status, determination of 25 hydroxy vitamin D in serum was assayed using a commercially available EIA kit (Epitope Diagnostics, Inc. San Diego, CA 92121, USA) according to manufacturer's instructions (19). Malondialdehyde (MDA) was measured as an indicator of lipid peroxidation status in all autistic children and control subjects according to the method described by Chauhan et al(20).

The study was approved by the Ethical Committee of the National Research Centre in accordance with the Declaration of Helsinki protocols. In addition, an informed written consent was signed by the parents or the legal guardians of the studied subjects.

Statistical analysis

Data were analysed using SPSS for Windows (ver.20.0) computing program. Distribution of the groups were analysed with one sample Kolmogrov–Smirnov test. As both groups showed normal distribution, parametric statistical methods were used. Results are presented as mean ±standard deviation. P value < 0.05 was considered significant.

RESULTS

The demographic data of patients and controls are presented in Table 1. No statistically significant difference was observed in age and sex between patients and controls. Within the patient group, the severity of autistic features was assessed by CARS. The age range for the autistic children was from 4-16 years (mean 5.6±3.2). Overall, the CARS scores for ASD ranged from 15 to 60, and the cut-off point for an autism diagnosis is a score above 30. According to the scoring standards of CARS, scores between 30 and 37 indicate mild to moderate autism and scores between 38 and 60 are characterized as severe autism.

The biochemical characteristics are presented in Table 2, showing significant increase of BDNF (ASD: 74.32±10.28ng/mL, Controls: 41.33±6.97ng/mL) and MAD in autistic children, while significant decrease of 25-hydroxy vitamin D levels in ASD compared to controls was noted. A positive correlation between the BDNF and CARS in children with ASD was detected ($r=0.72$, $p < 0.01$).

Table 1. The demographic data and clinical characteristics of the ASD patients and controls.

Variable	Patients (n=94)	Controls (n=73)
Age (mean ± SD, years)	5.6 ± 3.2	6.6 ± 2.2
Males [n (%)]	75 (79.8)	56 (76.7)
Females [n (%)]	19 (20.2)	17 (23.3)
Consanguinity [n (%)]	42 (44.7)	20 (27.4)
Similarly affected family members [n (%)]	30 (31.9)	-
Delivery problems [n (%)]	16 (17.02)	-
Delayed milestones [n (%)]	39 (41.5)	-
EEG [n (%)]	34 (46.8)	-
GIT [n (%)]	33 (35.1)	-
Sleep disorders [n (%)]	19(20.2	-
CARS		
Mild [n (%)]	22 (23.4	-
Moderate [n (%)]	11 (11.7	-
Severe [n (%)]	61 (64.9)	-

CARS: childhood autism rating scale, EEG: electroencephalogram, GIT: gastrointestinal tract

Table 2. Biochemical characteristics in ASD and controls.

Serum 25-hydroxy vitamin D (ng/mL)			
	ASD	**Controls**	**P value**
Min	10.11	25.91	-
Max	19.99	29.81	-
Mean± SD	18.22±4.09	28.0±8.12	0.006
Malonaldehyde (nmol/mL)			
	ASD	**Controls**	**P value**
Min	1.23	0.95	-
Max	2.78	1.82	-
Mean± SD	2.55± 0.64	1.35 ± 0.07	0.001
BDNF (ng/mL)			
	ASD	**Controls**	**P value**
Min	66.72	33.92	-
Max	77.84	45.81	-
Mean± SD	74.32±10.28	41.33±6.97	0.001

DISCUSSION

Autism spectrum disorders (ASD) is an assembly of developmental disability and neurological disorders that produces problems with feeling, thinking, language and communication. In our study there were 75 males and 19 females diagnosed as ASD, with a ratio of 3.9:1, male preponderance was delineated. Previous research (21) has reported that one of the most significant findings is the male preponderance with ASD and proposed that male vulnerability in ASD may be owing to environmental, genetic, or epigenetic purposes. A history of consanguineous marriage was present in 44.7% of the patients, which increases the risk for revealing recessive disorders and it is possible relationship for the development of ASD.

Therefore, consanguinity should be consistently screened when evaluating ASD (22). Similarly affected family members were reported in 31.9% of the patients. Sandin *et al* (23) found that genetic susceptibility was present in 50% of their patients and the risk of autism increased 10 times if a sibling had ASD and two-fold if a cousin had the diagnosis of ASM. The history taken from the mothers reported delivery problems in 17.02% of the patients. ASD children who were exposed to complications during birth, including preeclampsia, birth asphyxia were more prone to be ASD patients (24). To date, few prenatal or perinatal environmental factors have been specifically identified.

Developmental delay was reported in 41.5%, although (25) presented in his report that all children with ASD had delay in fine motor skills, gross motor skills or both. Another study by El-Bassyouni et al., 2013 elucidated that developmental delay was detected in 87.5% of their ASD patients. Moreover, 46.8% showed EEG variations, (27) noted the high prevalence of EEG abnormalities in children with ASD. On the other hand, Hara (28), documented that 18% of EEG changes occurred in ASD patients. 35.1% of the patients had gastrointestinal manifestations. Previous studies have described 9% to 70% frequency of gastrointestinal problems in children with autism (29; 30). Children with ASD present with sleep disturbances, in 50 and 80% (31). Although in our study only 20.2% of the patients had sleep disorders, this may be due to the difference in age between the studies as in early childhood, comparably high rates of sleep problems ASD patients are detected (32) (33). Most of the patients in our report showed severe degree of CARS scoring (46-60) in 64.9% of patients. Similarly, (34) described that 57% of their ASD patients had severe form of autism. Moreover, the malonaldehyde was significantly higher in patients than controls. This corroborates with the previous findings (2,7) reporting that ASD patients are vulnerable to oxidative stress.

Vitamin D levels were determined and showed a significant decrease in patients compared to control. Our results are consistent with the previous studies (35) and (9) reporting deficiency of Vitamin D in ASD children and recommended supplementing the patients with Vitamin D. Vitamin D is a neurosteroid that plays a crucial role in the development and function of the brain. Long-term vitamin D insufficiency prior to this important time of development may be a cause of abnormal brain maturation and function, and thus neurodevelopmental disorders such as ASD(36).

In our study there was significant increase in BDNF and decrease of Vitamin D compared to control. Because autism is a neurodevelopmental disorder that begins in childhood and BDNF plays an important role in neurodevelopment, its investigation as a sub-diagnostic biological marker of autism may lead autism researchers in a new direction of research and the development of effective treatment modalities(37). Likewise, the meta-analysis of previous studies (1,38) emphasized the deficiency of BDNF in ASD patients. Furthermore, a positive correlation between the BDNF and CARS in children with ASD was identified in our patients. This coincides with the findings of previous study (14). In contrast, other study (39) did not find a positive correlation between the BDNF and the CARS score. This may be owing to their limited number of studied patients.

In conclusion, BDNF levels can be deliberated as a prognostic or diagnostic marker of ASD. Our study sheds light on the importance of supplementation of ASD patients with antioxidants and vitamin D as it may help in the amelioration of their symptoms. Our findings point to a link between vitamin D and ASD in children. Vitamin D levels must be monitored in autistic children, particularly adolescents, in order to take preventative steps and treat the disorder early.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR INFORMATION

Moushira Zaki PhD, Professor of Human Genetics[1]
Hala T. El-Bassyouni, MD, Professor of Clinical Genetics[2]
Khaled Hamed, MD, Professor of Clinical Genetics[2]
Rehab Selim Ismail Moustafa, MD, Professor of Child Health[3]
Eman R. Youness, MD, Professor of Medical Biochemistry[4]

[1]Biological Anthropology Department, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt
[2]Clinical Genetics Department, Human Genetics and Genome Research Institute, National Research Centre, Cairo, Egypt
[3]Health Child Department, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt
[4]Medical Biochemistry Department, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt

CORRESPONDING AUTHOR

Moushira Zaki
Email: moushoraz@yahoo.com

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