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Original Article

Biochemistry Section

Evaluation of Iron Status in Children with Autism Spectral Disorder: A Case-control Study

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ABSTRACT

Introduction: Iron is an important factor in neural development. Iron Deficiency (ID) and Iron Deficiency Anaemia (IDA) anaemia is highly prevalent in patients of autism. There are paucity of studies to show association between iron profile and autism.

Aim: To investigate factors affecting iron status such as haemoglobin (%), serum iron, ferritin, and Total Iron Binding Capacity (TIBC) level in children with Autism Spectral Disorder (ASD) and healthy control.

Materials and Methods: It was a case-control study done from April 2018 to April 2019. Total 100 participants were recruited of which 50 autistic patients were taken as cases, and 50 healthy subjects were taken as control. Childhood Autism Rating Scale (CARS) was used to evaluate the severity of autistic symptoms. Cut-off value of serum ferritin was <10 ng/mL for preschoolers (<6 years) and <12 ng/mL for school-aged (>6 years) children to evaluate ID. Anaemia was defined as haemoglobin <11.0 g/dL for preschoolers and <12.0 g/dL for school-aged categorical variables and were compared by using Chi-square test. Normally distributed parametric variables were compared between groups by using independent samples t-test. Serum ferritin, iron, TIBC values were compared between severe, mild-moderate and control groups with Analysis of Variance (ANOVA). The p-value <0.05 was accepted to be statistically significant.

Results: Mean serum levels of ferritin, iron, TIBC were significantly reduced in ASD patients (p<0.001). The level of haemoglobin was also lower in ASD patients but it was not significant (p-value=0.51). Risk of ID and IDA was higher than normal subjects (RR for ID 1.74). Level of serum ferritin, iron and haemoglobin was lowest in severe autism as compared to mild-moderate autism and control groups.

Conclusion: These findings suggest iron and ferritin levels should be measured in autistic patients as a baseline investigation and it may be used as a screening test for ASD.

Keywords: Ferritin, Iron deficiency anaemia, Total iron binding capacity

INTRODUCTION

Autism Spectrum Disorder (ASD) is a group of disorders which is characterised by impairment in three area of developmental. The three areas are low socialisation, defective talking skills, restricted and repetitive behaviours [1,2]. These symptoms are often associated with a need for sameness, which leads to some feeding issues, such as mealtime rituals and a limited food repertoire [3]. Autism is a neurodevelopmental disorder that affects the multiple domains of development which includes language, sensory, motor function, social behaviour and their interests. While the aetiology of ASD is complex and not fully understood. A strong genetic evidence is suggested from twin and family history [4].

Asperger Syndrome, is towards upper side of spectrum in ASD, where symptoms are less severe. Patient has average intelligence and very few language problems. The lower end of spectrum consists of patients with classical autism in which symptoms are very severe. They have very poor socialisation and understanding of verbal and non-verbalcues. They also despise contact with human [5]. It typically affects the cerebral cortex, basal ganglia, corpus callosum, cerebellum, brain stem hippocampus and amygdala which are involve in higher mental function movement, behaviour, coordination, muscle for speaking and short-term memory. Hyperactivity, restrictive repetitive behaviour, mood problems, tics, self-injury are very common manifestation associated with autism [6]. Incidence of autitsm has increased significantly after 1980 [7]. The global prevalence of ASD at 0.76%; this accounts for approximately 16% of the global child population [8]. ASD is more common in males, male to female ratio of ASD is 4.3:1 [7]. Iron is an important neurotransmitter that acts as co-factor for enzymes which has role in cognitive, behavioural, and motor development [9]. Changes in serotonergic and dopaminergic systems, cortical networks, and myelination is seen when reduction in iron level is seen [10]. So, in ID, learning, memory, and psychomotor sensory functions attention is very much afflicted [10].

ID affects 47% of children worldwide: 50% of children in developing countries are affected with ID [11] and 6-12% of children in developed countries are affected [12]. The current parameters used for the diagnosis of ID include serum ferritin <10 µg/L [13]. There is a bidirectional relationship between ID and developmental problem such as autism. ID impairs the processes of neurotransmitter metabolism and myelin formation as well as altering energy metabolism in the brain. These affects are known to cause behavioural and cogitative developmental delay in children [14]. It is not clear that what is the degree and duration of ID which causes these developmental delays. There has been debate as to whether ID without anaemia may have any significant affect or not [15,16]. There are limited literature about the association between ID parameters and clinical symptoms of autism [1-3]. So, this study aimed to investigate factors affecting iron status such as haemoglobin (Hb), serum iron, ferritin, TIBC level in children with ASD compared to healthy control and to describe the degree of iron depletion and ID in children with autism.

MATERIALS AND METHODS

This was a case-control study conducted in the Department of Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna, India from April 2018 to April 2019. Cases of autism were recruited from Outpatient Department of Psychiatry (OPD). This study was approved by Institutional Review Board Research Ethics Committee of IGIMS, Patna letter no 417/IEC/2018/IGIMS. It has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

A series of interview was conducted and autism was diagnosed by a psychiatrist by gathering information of history, clinical feature, observation and CARS checklist [17]. Fifty patients of autism were

diagnosed according to criteria laid in Diagnostic and Statistical Manual of mental disorder (DSM) fourth edition [18]. Severity of autism was assessed by CARS checklist score. Written informed consent was taken from parents of participants.

Inclusion criteria: Patients aged 2-18 years, diagnosed case of autism, patient not receiving iron supplementation, patient not having any acute or chronic infection.

Exclusion criteria: Patients having any acute and chronic infection was excluded from study because ferritin is an inflammatory marker. Patient on iron supplementation, patient with hypoproteniemia and cancer were also excluded from the sudy.

Control group included 50 children, who referred to the Department for Counseling about child development, school adjustment and performance, teenage problems, family and friend relations, were recruited. They have no ASD according to DSM IV criteria. Age of the control subjects was between 2-18 years. They were selected after matching for the age with the cases to give a good representative sample of the population studied.

Childhood Autism Rating Scale (CARS) Checklist

This scale access behaviour in 14 domains that are generally applied in autism along with one general category with aim of identifying children with other developmental disorder. All items contribute equally to total score. Each item is scored between I to 4. A score below 30 classify children as normal. A score above 30 classifies autism. Autism is further classified into mild-moderate autism and severe autism with CAR score 30 to 36.5 and 37 and above, respectively [19].

Laboratory Measurement

Five mL blood was collected and sent to laboratory for analysing serum ferritin iron, TIBC and haemoglobin level.

Serum Iron and TIBC

Serum Iron was done by TPTZ (2,4,6-Tri-(2-pyridyl)-5-triazine) method [20] and TIBC was done by Nitroso-PSAP [21] method by AU5800. Reference range for iron was 0-4-month 110-270 µg/dL, 5-30 month 30-70 µg/dL, 24 month - 35-month 20-124 µg/dL, 3 to 11 year 53-119 µg/dL. Reference range for TIBC was 0-11 month 100-400 µg/dL, 12 month and older 250 -425 µg/dL [22].

Serum Ferritin

Serum ferritin lacks a uniform reference range in the literature. Because of variability in cut points for low values, a cut-off of <12 µg/L was used as suggested by the World Health Organisation (WHO) [23]. Serum ferritin was done by CLIA method by Beckman Coulter Access 2 [24].

Haemoglobin

Haemoglobin was done by Drabkins method [25].

Determination of Iron Deficiency (ID) and Iron Deficiency Anaemia (IDA)

Serum ferritin level is taken as an indicator of ID, because serum ferritin level reliably shows iron levels in body tissues including brain and is also an early precursor of ID. Ferritin cut off was <10 ng/mL for preschool children and <12 ng/mL for school-aged children to evaluate ID [26]. Anaemia was defined when serum ferritin was <12 ng/dL and level of haemoglobin was <11.0 g/dL for preschool children and <12.0 g/dL for school-aged children and adult [2].

STATISTICAL ANALYSIS

Data are expressed as arithmetic mean, and Standard Deviation (SD) unless otherwise stated. Student's t-test was used to ascertain the significance of differences between mean values of two continuous variables. Chi-square tests were performed to test for differences in proportions of categorical variables between two or more groups. One-way Analysis of Variance (ANOVA) was used when a categorical independent variable with two or more groups and a normally distributed interval dependent variable has to be to test for differences in the means of the dependent variable. The level p<0.05 was considered as the value for significance. Data were analysed by using Statistical Package for Social Sciences (SPSS) version 16.0.

RESULTS

In present study, total 100 participants were recruited out of which 50 patients of autism were taken as cases and 50 healthy participants were designated as control. Mean age of autistic verses control children were 8.51±4.72 year verses 10.27±4.11 years. Among cases, 43 (86%) were males and 7 (14%) were females. In control, 42 (84%) were males and 8 (16%) were females. Seventeen (34%) patients out of 50 have severe autism with CARS score more than 36.5 while 33 (66%) patients out of 50, have mild-moderate autism with CARS score between 30-36.5.

Baseline chemistry biomarker of autistic and control group was as follows: mean value of serum ferritin in autistic children was 30.89±15.79 µg/L and was significantly much lower than in control group 82.4±18.3 µg/L (p<0.001). Level of iron was also significantly lower in autistic group as compared to control group 61.54±19.95 verses 89.31±16.71 µg/dL (p<0.001), similarly study revealed that mean haemoglobin concentration was lowered in autistic group but difference is not significant (p-value=0.511) with control group. Mean TIBC is significantly high in autistic group [Table/Fig-1].

Parameters	Cases	Control	p-value
Serum ferritin (µg/L)	30.89±15.79	82.4±18.35	0.001
Serum iron (µg/dL)	61.54±19.95	89.31±16.71	0.001
Serum TIBC (µg/dL)	433.58±77.09	344.86±39.15	0.001
Haemoglobin (g/dL)	10.34±1.18	12.05±1.15	0.511

[Table/Fig-1]: Level of Iron Deficiency (ID) parameters in case and control.
p-value was calculated using independent sample t-test; p-value less than 0.05 was considered statistically significant

The relative risk of IDA in autistic cases as compared to control was 1.70 and there was significant difference between groups. Whereas, relative risk of ID was 1.74 in case of autism [Table/Fig-2].

Parameters	Cases	Control	RR	p-value
Iron deficiency anaemia	13	4	1.70	0.004
Iron deficiency	19	7	1.74	0.001

[Table/Fig-2]: Relative risk of Iron Deficiency (ID) and Iron Deficiency Anaemia (IDA) in case and control.
RR: Relative risk, Chi-square test was used, p-value <0.05 considered significant

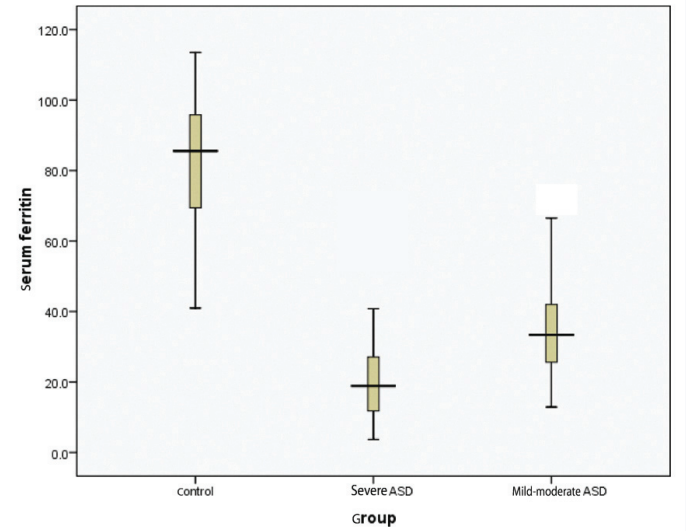
Comparison of serum ferritin, iron, TIBC, and haemoglobin among three groups i.e., severe autism, mild-moderate autism and control group demonstrated that ferritin, iron, TIBC and haemoglobin level were different between three groups. Analysis indicated that mean level of ferritin in severe autism group is lowest as compared to mild-moderate and control group (p<0.001). The result also showed that mean level of iron and haemoglobin is also lowest in severe autism group [Table/Fig-3].

Box plot illustrating distribution of ferritin in three different groups. Median of ferritin in control group is highest followed by mild-moderate and severe ASD showing difference in these groups. There were two outliers in severe ASD and one outlier in mild-moderate ASD [Table/Fig-4].

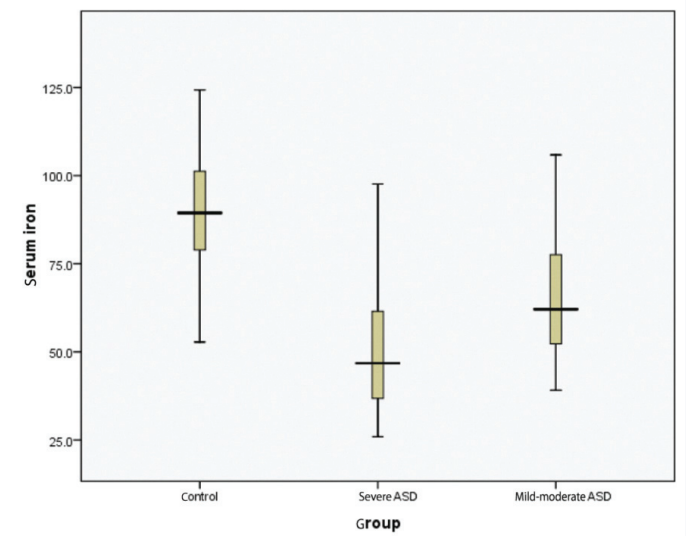
Box plot showing distribution of iron level in control, severe ASD and mild-moderate ASD group. Median is highest in control group as compared to mild-moderate and severe ASD group [Table/Fig-5]. Box plot illustrating the distribution of TIBC in three different groups. Here comparison of median also showed difference in all the three groups [Table/Fig-6].

Parameter	Group	Mean	SD	F	p-value
Ferritin	Severe ASD	23.07	16.64	121.628	0.001
	Mild-Moderate ASD	34.91	13.93		
	Control	82.47	18.35		
Iron	Severe ASD	52.04	20.27	33.924	0.001
	Mild-Moderate ASD	66.44	18.21		
	Control	89.31	16.71		
TIBC	Severe ASD	484.71	79.67	42.833	0.001
	Mild-Moderate ASD	407.24	61.86		
	Control	344.86	39.15		
Haemoglobin	Severe ASD	10.17	1.24	26.853	0.001
	Mild-Moderate ASD	10.42	1.18		
	Control	12.03	1.67		

[Table/Fig-3]: Comparison of Iron Deficiency (ID) parameters (Ferritin, Iron, TIBC and Haemoglobin) in severe autism, mild-moderate autism and control group.
p-value was calculated using ANOVA; p-value <0.05 was considered statistically significant



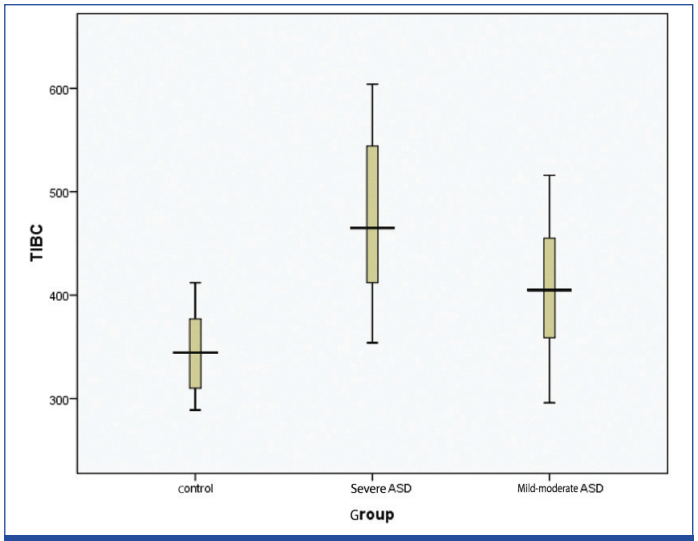
[Table/Fig-4]: Distribution of ferritin in severe, mild-moderate, control group.



[Table/Fig-5]: Distribution of serum iron level in different groups.

DISCUSSION

Level of mean serum ferritin in present study was significantly lower in autistic cases as compared to control. This finding was supported by some previous study from South Wells, Turkey, Canada that reported higher rate of ferritin deficiency in autistic children then in normal population. Pakyurek M et al., found decreased level of serum ferritin in autistic children in California [26]. Bener A et al., found very low serum ferritin in ASD 35.7±5.12 ng/mL verses 38.49±5.73 ng/mL in control patient in Turky [27]. Bilgiç A et al., also demonstrated low



[Table/Fig-6]: Distribution of serum TIBC level in different groups

serum ferritin in autistic patient in Turkish population [28]. Dosman CF et al., reported baseline ferritin level 15.72 µg/l (4.2-39.0 µg/L) in cases of autism [29], which was increased to 28.8 µg/L (6.6- 103 µg/L) after iron supplementation in Toranto, Canada. Latif A et al., in South Wells, found high prevalence of ferritin and ID 52% in children with autism [30]. Herguner S et al., significantly low level of ferritin iron haemoglobin in children suffering from autism. He also found positive correlation between age and ferritin deficiency [2]. Bener A et al., also reported very low level of ferritin (36.75±5.12 ng/dL vs 38.49±5.73 ng/mL) in autistic children as compared to control in population of Qatar [31]. Chen MH et al., in Taiwan and Deth R et al., in Boston, USA also reported low level of ferritin in autistic children [32,33].

Prevalence of ID and IDA in this study was 38% and 26% respectively in patients of autism. Several studies reported association of ID and IDA with autism [27,34,35]. Herguner S et al., declared that ID was more common in preschool children with autism [2]. In this study, 24.1% of autistic children had ID and 15.5% had IDA. Bilgiç A et al., reported that the prevalence of ID in autistic children under 6 years was higher than autistic children over 6 years and ID was found in 32% of preschool autistic children [28]. In a study in South Wells investigating iron levels in autism patients between 19 months and 13 years, ID was seen in 52% of children with autism. He also found 52% children with ASD iron deficient compared with 13.6% with asperger syndrome [30].

The present study found low mean value for serum iron also which was in parallel with some previous studies. Bener A et al., in his study in Qatar found mean values of serum iron in autistic children was significantly much lower than the normal value in the control children [31]. Studies by Herguner S et al., and Bilgiç A et al., also found low mean level of iron in children suffered with autism in Turkeish population [2,28]. In ASD it has been seen that children have limited preference to food and the develop picky eating habit and very resistant to eat. This supports decrease level of mean iron in this group [34]. As gastrointestinal symptoms are frequent in children with autism [36], impaired absorption might be another possible cause of ID. However, Dosman CF et al., reported that ferritin and iron levels increased after iron supplementation which excludes the probability of malabsorption [29].

As iron has very important role in cognitive, motor sensory, social and emotional development. It functions is very important in development of central nervous system because enzyme and protein used in this process requires iron. Iron also plays an important part of serotonergic, dopaminergic pathways and in myelination of neurons [28]. In addition, a genetic predisposition to ID in ASD has also been proposed [13]. So, ID increases the risk of psychiatric disorder including mood disorder, ASD and attention deficit hyperkinetic disorder [30].

In this present study, level of iron, ferritin and haemoglobin was lowest in severe autism. Finding of this study was parallel with a study conducted by Gunes S et al., who reported low ferritin and iron in severe anaemia compared to mild-moderate anaemia [37]. The present study showed that there is an association between ASD with ID and IDA. decreased levels of serum ferritin, in children with ASD might be a sign of ID and it may be a cause of IDA.

Limitation(s)

First limitation was low sample size hence generalisation of result on population was not possible. Second was authors did not conduct a food survey to record diet of autistic children as most common cause of ID is inadequate diet intake.

CONCLUSION(S)

Low level of serum ferritin and high prevalence of IDA in autistic disorder suggests possible deficiency of iron in causation of autism. Significantly lower level of iron and ferritin in severe autism shows some link between iron level and severity of symptoms of ASD. This finding suggests that iron and ferritin level should be monitored in patients of autism as baseline investigation and can be used as a screening marker to ameliorate severe symptoms in patients of autism. However, extensive studies should be done with randomised control trial to prove serum ferritin and iron as an interventional factor.

REFERENCES

[1] Reynolds A, Krebs NF, Stewart PA. Iron status in children with autism spectrum disorder. Pediatrics. 2012;130:154-59.
[2] Herguner S, Kelesoglu FM, Tanidir C. Ferritin and iron levels in children with autistic disorder. Eur J Pediatr. 2012;171:143-46.
[3] Al-Ali SF, Alkaissi A. Association between autism spectrum disorder and iron deficiency in children diagnosed autism spectrum disorder in the northern west bank. J Health Med Nur. 2015;16.
[4] Gupta AR, State MW. Recent advances in the genetics of autism. Biol Psychiatry. 2007;61(4):429-37.
[5] Howlin P. Autism spectral disordar. Psychiatry. 2016;5(9):320-24.
[6] Castine AWH, Nault K, Green G. An assessment of food acceptance in children with autism or pervasive developmental disordar-Not otherwise specified. J Autism Dev Disord. 2001;31(5):505-11.
[7] Newschaffer CJ, Croen LA, Daniels J. The epidemiology of autism spectrum disorders. Annu Rev Public Health. 2007;28:235-58.
[8] Baxter AJ, Brugha TS, Erskine HE, Scheurer RW, Vos T, Scott JG. The epidemiology and global burden of autism spectrum disorders. Psychol Med. 2015;45:601-13. 10.1017/S003329171400172X.
[9] Erikson KM, Jones BC, Hess EJ. Iron deficiency decreases dopamine D1 and D2 receptors in rat brain. Pharmacol Biochem Behav. 2001;69(4):409-18.
[10] Grant CC, Wall CR, Brewster D. Policy statement on iron deficiency in pre-school-aged. Paediatr Child Health. 2007;43:513-12.
[11] American Psychiatric Association (APA). Diagnostic and Statistical Manual of Mental Disorders. 4th ed. Washington: American Psychiatric Association; 1994.
[12] Moy RJ. Prevalence, consequences and prevention of childhood nutritional iron deficiency: A child public health perspective. Clin Lab Haematol. 2006;28:291-98.
[13] Beard J. Iron deficiency alters brain development and functioning. J Nutr. 2003;133(5Suppl.1):1468S-72S.
[14] Carter RC, Jacobson JL, Burden MJ, Armony-Sivan R, Dodge NC, Angelilli ML, et al. Iron deficiency anemia and cognitive function in infancy. Pediatrics. 2010;126(2):e427-34.
[15] Metallinos-Katsaras E, Valassi-Adam E, Dewey KG, Lonnerdal B, Stamoulakatou A, Pollitt E. Effect of iron supplementation on cognition in Greek preschoolers. Eur J Clin Nutr. 2004;58:1532-42.
[16] Zimmermann MB. Methods to assess iron and iodine status. Br J Nutr. 2008;99(suppl 3):S02-09.
[17] Love SR. The Childhood Autism Rating Scale, 2nd ed. Torrance, CA: Western Psychological Services, Inc; 2010.
[18] American Psychiatry Association. Diagnostic and Statistical Manual of Mental Disorders, 5th ed. Arlington, VA: American Psychiatric Publishers; 2013:19.
[19] Chakraborty S, Thomas P, Bhatia T, Vishwajit L, Smita N, Deshpande N. Assessment of severity of autism using the Indian scale for assessment of autism. Indian J Psychol Med. 2015;37(2):169-74.
[20] Frederic Smith G. The Iron Reagents, 3rd ed. Chapter IV, G Fredrick Smith Chemical Company; 1980. 31-40.
[21] Yue Y, Arana C, Staples M. Development of a fully auto-mated TIBC method for the DimensionÛ clinical chemistry system [Abstract]. Clin Chem. 2000;46:A146.
[22] National Committee for Clinical Laboratory Standards. Determination of serum iron and total iron binding capacity; proposed standard. NCCLS document H17-P, Vol. 10, No. 4. Wayne, PA: NCCLS, 1990.
[23] World Health Organization. Assessing the Iron Status of Populations: Report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level. Geneva, Switzerland: World Health Organization, Centers for Disease Control and Prevention; 2005.
[24] Formen DT, Parker SL. The measurement and interpretation of serum ferritin. Ann Clin Lab Sci. 1980;10(4):345-50.
[25] Srivastava T, Negandhi H, Neogi SB, Sharma J, Saxena R. Methods for haemoglobin estimation: A review of "what works" J Hematol Transfus. 2014;2(3):1028.
[26] Pakyurek M, Azarang A, Maria A, Thomas L, Nordahl E. Assessment of biometal profile in children with autism spectrum disorder, with attention deficit hyperactivity disorder, or with other psychiatric diagnoses: A comparative outpatient study. Actapsychologica. 2018;4:6.
[27] Bener A, Khattab A, Bhugra D, Georg F, Hoffmann Y. Vitamin D levels among autism spectrum disorders children. Ann Afr Med. 2017;16(4):186-91.
[28] Bilgiç A, Gürkan K, Türkoğlu S. Iron deficiency in preschool children with autistic spectrum disorders. Res Autism Spectr Disord. 2010;4:639-44.
[29] Dosman CF, Drmic IE, Brian JA, Senthilselvan A, Harford M, Smith R. Ferritin as an indicator of suspected iron deficiency in children with autism spectrum disorder: Prevalence of low serum ferritin concentration. Dev Med Child Neurol. 2006;48(12):1008-09.
[30] Latif A, Heinz P, Cook R. Iron deficiency in autism and asperger syndrome. Autism. 2002;6(1):103-14.
[31] Bener A, Khattab AO, Al-Dabbagh MM. Is high prevalence of Vitamin D deficiency evidence for autism disorder?: In a highly endogamous population. J Pediatr Neurosci. 2014;9(3):227-33.
[32] Chen MH, Su TP, Chen YS, Hsu JW, Huang KL, Chang WH. Association between psychiatric disorders and iron deficiency anemia among children and adolescents: A nationwide population-based study. BMC Psychiatry. 2013;13:161. Doi: 10.1186/1471-244X-13-161.
[33] Deth R, Muratore C, Benzecry J, Power-Charnitsky VA, Waly M. How environmental and genetic factors combine to cause autism: A redox/methylation hypothesis. Neurotoxicology. 2008;29(1):190-201.
[34] Dosman CF, Brian JA, Drmic IE. Children with autism: Effect of iron supplementation on sleep and ferritin. Pediatr Neurol. 2007;36(3):152-58.
[35] Szatmari P, Jones MB, Zwaigenbaum L, MacLean JE. Genetics of autism: Overview and new directions. J Autism Dev Disord. 1998;28(5):351-68.
[36] Valicenti-McDermott M, McVicar K, Rapin I, Wershil BK, Cohen H, Shinnar S. Frequency of gastrointestinal symptoms in children with autistic spectrum disorders and association with family history of autoimmune disease. J Dev Behav Pediatr. 2006;27(2suppl):S128-36.
[37] Gunes S, Ekinci O, Celik T. Iron deficiency parameters in autism spectrum disorder: Clinical correlates and associated factor. Italian Journal of Pediatrics. 2017;43:86.

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