Paediatrica Indonesiana

p-ISSN 0030-9311; e-ISSN 2338-476X; Vol.61, No.2(2021). p.82-8; DOI: 10.14238/pi61.2.2021.82-8

Original Article

Hypovitaminosis D as a risk factor for severe autism spectrum disorder

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Abstract

Background Vitamin D is an important risk factor for autism spectrum disorder (ASD). However, research on hypovitaminosis D as a risk factor for severe ASD has been limited. To our knowledge, no such studies have been done in Indonesia.

Objective To evaluate hypovitaminosis D as a risk factor for severe ASD.

Methods This cross-sectional study included children aged 2-18 years who fulfilled the ASD DSM-5 diagnostic criteria. Subjects were consecutively sampled from April - June 2019 at the Child Growth and Polyclinic, Dr. Sardjito General Hospital, Yogyakarta. Assessment of ASD severity was carried out using the *Childhood Autism Rating Scale Second Edition* (CARS-2) questionnaire. Serum 25(OH)D examination was done in the Clinical Laboratory, Dr. Sardjito General Hospital.

Results Of 36 children with ASD, 36.1% had hypovitaminosis D (<30 ng/mL) and 69.4% had severe ASD, based on the CARS-2 questionnaire (≥37-60). Bivariate analysis revealed that children with hypovitaminosis D had more severe CARS-2 values (92.3%) compared to those with normal vitamin D levels (56.5%) (PR 1.633; 95%CI 1.10 to 2.42; P=0.031). Multivariate analysis with logistic regression revealed that hypovitaminosis D increased the risk of severe ASD (PR 1.65; 95%CI 1.06 to 2.56; P=0.037). However, other variables such as gender, parental education, attention deficit and hyperactivity disorder (ADHD), epilepsy, sleep disorders, pharmacotherapy and non-pharmacotherapy had no significant relationships with severe ASD.

Conclusion Children with ASD and hypovitaminosis D have a 1.65 times higher risk of severe ASD compared to children with ASD and sufficient vitamin D levels. We recommend that children with ASD undergo serum 25(OH)D monitoring. **[Paediatr Indones. 2021;61:82-8 ; DOI: 10.14238/pi61.2.2021.82-8**].

Keywords: *autism spectrum disorder, vitamin D, CARS-2*

Autism spectrum disorders (ASD) are a complex neurodevelopmental condition including disruption in social interactions and communication, accompanied by stereotypic and repetitive movements with varying degrees of severity.[1] Over the past few decades the number of ASD patients in the US has increased dramatically, based on *Centers for Disease Control and Prevention* (CDC) report which estimated 1 in 68 children had ASD in the United States.[2,3] In 1992, the number of ASD patients in Indonesia was known to be 1 in 833 children, but to date, the lack of autism study has resulted in a dearth of information. Nonetheless, a 2017 report suggested that the number of ASD patients is increasing.[4] Four to five times more boys than girls have ASD.[5] Other risk factors for autism are a family member with autism, and monozygotic twins compared to dizygotic twins.[6]

The etiology of ASD is complex, multifactorial, and can only be identified in 15-20% of patients.

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Submitted March 10, 2020. Accepted March 2, 2021.

Genetic and environmental factors are suspected to have roles in the ASD etiology.[7,8] One such environmental factor is low vitamin D levels.[2,6,7,9] Vitamin D has important roles in the immune system as well as in homeostasis in the central nervous system.[2,7,10]

Individuals suspected of having ASD need a comprehensive assessment based on the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5). A diagnosis of ASD must fulfill the DSM-5 criteria A1 to A3 (permanent deficits in social communication and social interaction in all contexts marked by history or present circumstances), B1 (motion, use of objects or stereotypic / repetitive speech), B4 (hyperreactive or hyporeactive to sensory input or unusual interest in environmental sensory aspects); and D (symptoms cause forced disruption in social functions, work or other fields). In addition, DSM-5 can be used to assess ASD severity, divided into three levels.[1] The severity levels are based on the extent of support needed to perform developmental tasks. More specific tools to measure autism severity based on the core symptoms of autism are available, such as the *Childhood Autism Rating Scale Second Edition* (CARS-2), which is used in Indonesia. Several studies have reported that CARS-2 has good sensitivity (94%) and specificity (85%).[11-13]

Previous studies have suggested that low vitamin D levels are associated with ASD,[3,6,7,9,14-19] and negatively correlated with ASD severity.[3,20] In contrast, other studies have found no relationship between low vitamin D level and pediatric ASD.[14,21-24] Indonesia is a tropical country with plenty of sun exposure, but the prevalence of children with low vitamin D status is quite high.[25] To date, children with ASD do not routinely undergo vitamin D examination and/or supplementation. Hence, we aimed to evaluate hypovitaminosis D as a risk factor for severe ASD in children.

Methods

This cross-sectional study was done in Yogyakarta children aged 2-18 years, who had been diagnosed with ASD based on DSM-5 criteria. Subjects had been diagnosed by Pediatric Specialists in the Growth, Development, and Social Pediatrics Subdivision at the Child Growth and Polyclinic, Dr. Sardjito General Hospital, Yogyakarta. Consecutive sampling was done from April to June 2019 until a minimum sample size of 36 subjects was met. Children who had received vitamin D supplementation were excluded.

The primary data collected were patient identity, age, gender, parental education level attained (primary to high school as low education and bachelor's or equivalent as high education), ASD diagnosis based on DSM-5, comorbidities such as attention deficit and hyperactivity disorders (ADHD) (diagnosed using DSM-5), sleep disorders (using the *Sleep Disorders Scale Questionnaire*/SDSC), history of epilepsy [by electroencephalographic (EEG) results], as well as various therapies including behavioral, occupational, speech, and medical in the form of risperidone, aripiprazole, anti-epilepsy medication, methylphenidate, and/or melatonin. Parents provided written informed consent. The CARS-2 questionnaire for ASD severity consisted of 14 questions, answered by parents' reports, observations, and medical records relevant to assessing the behavior of children suspected of having autism, as well as one question for the assessor on a general description of the child suspected to have autism. Each question had a value ranging from 1 to 4, with higher scores indicating increasing severity. The total CARS-2 value ranged from 15 to 60, with scores of 30 to 36.5 indicating mild to moderate autism, and scores of 37 to 60 indicating severe autism. The assessor/observer was a pediatrician. Serum 25(OH)D levels were measured by Chemiluminescent Immunoassay (CLIA) at Dr. Sardjito General Hospital Clinical Laboratory.[26,27] Classification of serum 25(OH)D levels according to *Endocrine Society* divided into vitamin D deficiency (<20 ng/mL), vitamin D insuffiency (21-<30 ng/mL) and vitamin D sufficiency (≥ 30 ng/mL).[28] In this study, deficient and insufficient were defined as hypovitaminosis D.

Bivariate and multivariate analyses were done with *SPSS version 22* for *Windows*, using Chi-square or Fisher's exact test, where appropriate. The relationship between variables and outcome was indicated by the prevalence ratio (PR), with 95% confidence interval (CI). This study was approved by The Ethics Committee in Department of Child Health, Faculty of Medicine, Public Health, and Nursing Univertas Gadjah Mada.

Results

We conducted a study of hypovitaminosis D as a risk factor for severity in ASD children at Dr. Sardjito General Hospital, Yogyakarta. Distribution of study subjects based on age, sex, parental education, vitamin D level profiles, comorbidities such as ADHD, epilepsy and sleep disorders, medical and non-medical therapy. The mean age of subjects was 5.82 (SD 3.23) years, with 30/36 boys. Most subjects' parents had attained a bachelor's degree or the equivalent (25/36 fathers and 19/36 mothers). Hypovitaminosis D was found in 13/36 (subjects while normal or sufficient vitamin D levels were found in 23/36. According to the CARS-2 results, the majority of subjects had severe ASD (25/36 subjects) and 11/36 had mild-moderate ASD. Subjects' co-morbidities were ADHD (9 subjects), epilepsy (6 subjects), and sleep disorders (32 subjects). Subjects received various medical and non-medical therapies as follows: risperidone or aripiprazole (23 subjects), behavioral therapy (24 subjects), occupational therapy (32 subjects), and speech therapy (27 subjects) (**Table 1**).

Bivariate analysis with Fisher's exact test revealed that hypovitaminosis D was a significant risk factor for severe ASD (PR 1.63; 95%CI 1.10 to 2.42; P=0.031). Hypovitaminosis D was associated with a 1.63-fold higher risk of increased ASD severity compared to normal or sufficient vitamin D levels, as shown in **Table 2**.

As shown in **Table 3**, Fisher's exact test revealed no significant correlation between ADHD and ASD severity (PR 1.41; 95%CI 0.98 to 2.04; P=0.223). Furthermore, none of the other possible confounding factors had significant correlations with ASD severity. ADHD was the only variable with P value < 0.25, so ADHD and hypovitaminosis D were further analyzed by multivariate logistic regression, which revealed that only hypovitaminosis D had a significant correlation with ASD severity (PR 1.65; 95%CI 1.06 to 2.56; P=0.037). Children with ASD and hypovitaminosis D had a 1.65 times increased risk of severe ASD compared to those with sufficient vitamin D (**Table 4**).

Table 1. Basic characteristics of subjects

Characteristics	(N=36)
Mean age (SD), years	5.82 (3.23)
Gender, n	
Male	30
Female	6
Paternal education, n	
Low	11
High	25
Maternal education, n	
Low	17
High	19
Vitamin D level, n	
Hypovitaminosis (<30 ng/mL)	13
Sufficient (≥30 ng/mL)	23
CARS-2, n	
Mild-moderate (30-<37)	11
Severe (≥37-60)	25
ADHD, n	
Yes	9
No	27
Epilepsy, n	
Yes	6
No	30
Sleep disorder, n	
Yes (SDSC ≥ 39)	32
No	4
Medical therapy (risperidone and/or aripiprazole), n	
Yes	23
No	13
Behavioral therapy, n	
Yes	24
No	12
Occupational therapy, n	
Yes	32
No	4
Speech therapy, n	
Yes	27
No	9

Table 2. Analysis of hypovitaminosis D and ASD severity

Vitamin D level	CARS-2		PR	95%CI	P value
	Severe (n=25)	Mild-moderate (n=11)			
Hypovitaminosis, n	12	1	1.63	1.10 to 2.42	0.031
Sufficient, n	13	10			

Table 3. Bivariate analysis of confounding/external variables and ASD severity

Confounding/external variables		CARS-2				
		Severe (n=25)	Mild-moderate (n=11)	PR	95%CI	P value
Gender, n	Male	21	9	1.05	0.57 to 1.94	1.000
	Female	4	2			
Paternal education, n	Low	8	3	1.07	0.68 to 1.68	1.000
	High	17	8			
Maternal education, n	Low	13	4	1.21	0.78 to 1.87	0.481
	High	12	7			
Epilepsy, n	Yes	4	2	0.95	0.52 to 1.76	1.000
	No	21	9			
ADHD, n	Yes	8	1	1.41	0.98 to 2.04	0.223
	No	17	10			
Sleep disorder, n	Yes	23	9	1.44	0.53 to 3.92	0.570
	No	2	2			
Medical therapy, n	Yes	17	6	1.20	0.73 to 1.97	0.475
	No	8	5			
Behavior therapy, n	Yes	18	6	1.29	0.76 to 2.19	0.446
	No	7	5			
Occupational therapy, n	Yes	22	10	0.92	0.49 to 1.69	1.000
	No	3	1			
Speech therapy, n	Yes	19	8	1.06	0.63 to1.78	1.000
	No	6	3			

Table 4. Multivariate analysis of confounding/independent variables and ASD severity

Variables	PR	95%CI	P value
Vitamin D	1.65	1.06 to 2.56	0.037
Hypovitaminosis			
Sufficient			
ADHD	1.45	0.88 to 2.38	0.126
Yes			
No			

Discussion

The mean age of our 36 subjects (30 boys and 6 girls) was 5.82 (SD 3.23) years. The prevalence of ASD in boys is reportedly much higher than in girls, with a ratio of 4-5:1.[5] One hypothesis for this imbalance is that the X chromosome in girls confers protection from ASD.[29,30]

Low vitamin D levels have been hypothesized as an environmental risk factor in children with ASD,[7,8] as hypovitaminosis D from pregnancy to the beginning of life may have a cumulative effect.[8,31] In our study, 13/36 children with ASD had hypovitaminosis D. Of our subjects with hypovitaminosis D, 12/13 had severe CARS-2 scores, whereas only 12/23 of vitamin D sufficient subjects had severe CARS-2 scores. Similarly, a study showed that mean 25(OH)D in severe autism was significantly lower than in mild-moderate autism,[3] as did a study in Saudi Arabia.[20] These results possible link between level of vitamin D deficiency and the severe autism symptoms.

Hypovitaminosis D increased the risk of severe autism by 1.65 times compared to subjects with sufficient vitamin D and our study did not have a control group. A previous study reported that serum vitamin D levels were significantly lower in Chinese children with ASD compared with healthy children and an inverse relationship with ASD severity,[17] which was not compared in our study. The result supported hypothesis that lower serum vitamin D levels could be implicated severe autism.

Of male subjects, 21/30 had severe ASD, and of our female subjects, 4/6 had severe ASD. In contrast, a study stated that while the ASD prevalence was higher in boys than girls, girls had more severe ASD. They used *The Autism Diagnostic Interview, Revised* (ADI-R) questionnaire instead of the CARS-2

questionnaire used in our study. The relationship between sex and the ASD severity remains unclear.[32]

Of subjects with low paternal education (8/11) and low maternal education (13/17) had severe ASD. These results were not associated with severe ASD. Similarly, a previous study reported no relationship between parental education and ASD severity, and suggested that parental stress plays a dominant role in care that required extra attention and support due to lack of social skills, and may have an effect on ASD severity.[33] We did not evaluate parental stress levels.

Symptoms of ADHD are common comorbidities in children with ASD.[34] In our study, there was no significant association between ADHD and severe ASD. In contrast, a study which used 3 questionnaires to assess the severity of ASD, reported that ASD patients with ADHD had more severe ASD symptoms using the ADI-R and The Social Responsiveness Scale (SRS) questionnaires, but no significant difference using *The Autism Diagnostic Observation Schedule-Generic* (ADOS-G) questionnaire.[35] This previous study did not use the CARS-2 questionnaire to assess the severity of autism.

About 30% of autistic children have comorbid epilepsy, based on history of seizures and abnormal EEGs.[36] In this study, no significant association was found between epilepsy and severe ASD. However, two previous studies concluded that autistic children with epilepsy had more severe autism symptoms than those without epilepsy. These studies reported a direct relationship between ASD and epilepsy, namely, that intellectual disability (ID) which is more common in autistic children with epilepsy could affect ASD severity.[37,38] As we did not assess our subjects' intellectual ability, we were unable to analyze for such as association.

There is a fairly high prevalence of comorbid sleep disorders in ASD.[5,34] We assessed sleep disorders with SDSC questionnaire. There were more severe ASD cases in children with co-morbid sleep disorders than in those without (23/32 *vs.* 2/4, respectively), but the difference was not significant (P=0.57). In contrast, a study showed that a strong correlation between greater sleep disturbances have greater ASD severity with *Autism Spectrum Disorder-Diagnostic-Child* (ASD-DC) questionnaire.[39] They used a different questionnaire with our study.

Autistic spectrum disorder can be managed by medical and non-medical therapies.[5,40] While there are no specific treatments for the core symptoms of ASD, integrated management can be adjusted to address the symptoms and comorbidities for each child. The goals of these therapies is to maximaze the quality of life.[5] According to FDA recommendations, the most widely given medical therapies are atypical antipsychotics, namely, risperidone or aripiprazole.[41] In our study, there were more severe ASD subjects in our medically-treated group than those not medically treated (17/23 *vs.* 8/13, respectively), but the difference was not significant. In contrast, a study noted that risperidone or aripiprazole reduced the severity of autism, based on *Abberant Behavior Checklist* (ABC) questionnaire results. They used a different method and questionnaire with our study. There are many types of non-medical therapy that can be applied, but the choice of therapy depends on each ASD.[5,40] We subdivided into behavioral, occupational, and speech therapies. There were no significant differences in percentages of severe ASD in any of the non-medical therapy sub-groups compared to those who had not received non-medical therapies. Another study reviewed that there were no meaningful differences in the group that received interventions but related to the level of parental education.[42] Our study did not assess the relationship, nor can we conclusively determine if the therapies improved the ASD symptoms in our subjects.

A limitation of this study was its cross-sectional design, which cannot be used to determine causal relationships between variables. In addition, the CARS-2 questionnaire for ASD severity is subject to respondent recall bias and lack transparency. Furthermore, we did not assess variables that might affect vitamin D levels, such as exposure to sunlight, either for a long duration or in daily activities, parental mental health and stress, which could affect caregiving.

In conclusion, children with ASD and hypovitaminosis D have a 1.65 times higher risk of severe ASD compared to ASD children with sufficient vitamin D. Hence, we recommend that all ASD patients undergo serum 25(OH)D evaluations. In addition, ASD patients with hypovitaminosis D who take vitamin D supplements should be periodically reevaluated with regards to vitamin D levels and ASD severity.

Conflict of Interest

None declared.

Funding Acknowledgment

The authors received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

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