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Original Article

Hair zinc level and autism spectrum disorder in children

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Abstract

Background Autism spectrum disorder (ASD) is one of the most common neurodevelopmental disorders. In children with ASD, the neurodevelopmental and behavioral disorders are characterized by impaired quality of interaction, communication, and social imagination, with limited interest and repetitive behavior. Various studies have found that the mean zinc level is lower in children with ASD than in children without ASD, especially in the low functioning autism.

Objective To compare hair zinc level in children with and without ASD.

Methods This case-control study included children with ASD and controls without ASD. Subjects were children under 5 years old who came to Prof I.G.N.G. Ngoerah Hospital and Masadini Clinic in Denpasar. Hair zinc cut-off level was determined by the area under curve (AUC) based on the receiver operating characteristic (ROC) curve.

Results A total of 128 subjects consisted of 64 cases and 64 controls, with a mean age of 4 (SD 0.8) years. Most subjects were male (76%), 82.3% were well-nourished. The mean maternal age at delivery was 28.82 (SD 3.2) years. Subjects' mean hair zinc level was 87.9 (SD 231.1) μg/g. Bivariate analysis with Chi-square test revealed that children with hair zinc concentration of <33.88 μg/g were 22.19 times more likely to have ASD than those with higher zinc levels (OR 22.19; 95%CI 8.02 to 42.09; P<0.001).

Conclusion Low hair zinc level is more likely to occur in children with ASD than in children without ASD. **[Paediatr Indones. 2024;64:227-32; DOI: 10.14238/pi64.3.2024.227-32**].

Keywords: *autism spectrum disorder; children; hair zinc level*

Autism spectrum disorder (ASD) is a neurodevelopmental and behavioral disorder characterized by impaired quality of interaction, communication, and social imagination, with children exhibiting limited interest in their surroundings and repetitive behavior.[1] It is one of the most common neurodevelopmental disorders in children. The prevalence of ASD significantly increased from 1 in 150 children in 2002 to 1 in 88 children in 2008.[2]

Autism spectrum disorder pathogenesis and pathophysiology is still largely unknown. An imbalance of mineral elements in the body is thought to affect the central nervous system, leading to numerous health problems including development of ASD. Studies have shown that children with ASD experience poorer ability to remove metal toxins out of the body. Since zinc is crucial for neurological functioning and metal detoxification, deficiencies may contribute to the development of ASD. Zinc plays an important role in the metabolism of protein, carbohydrate, and fat.

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Zinc is also a co-factor of metalloenzymes and acts as a neurotransmitter or neuromodulator of the nervous system. Zinc has roles in synaptic transmission, endogenous neuromodulation, metabolism of nucleic acids, and growth of brain microtubules.[3,4]

In 2011, a study showed that children with ASD, especially low functioning autism, had lower mean hair zinc level when compared with children without ASD.[5] Another study found that of 78 children with ASD, 66 children had lower hair zinc level than expected.[6] Further supporting this finding, a previous study showed that children with ASD had lower zinc level in plasma, red blood cells, and hair compared to children without ASD.[7] People with zinc deficiency are also at risk of neuropsychological changes, such as unstable emotions, becoming more sensitive and depressed. Significant negative correlations between zinc level and fear and anxiety (P=0.022), as well as verbal communication (P=0.017).[7] Results have differed among studies. Another study showed that children without ASD had mean hair zinc level of 2.9 μg/g, while children with ASD had a mean 5.4 μg/g (P=0.0001).[8]

Measurement of hair zinc level in children with and without ASD has not been widely studied in Indonesia, especially in Bali. Zinc levels are commonly measured in serum, nails, and hair. Nails or hair can yield more stable results due to their having slower metabolism of the mineral than that of serum.[9] We aimed to compare hair zinc level in children with and without ASD.

Methods

This unpaired, case-control study had a 1:1 ratio of subjects with and without ASD who were seen in the Outpatient Department of Prof. I.G.N.G. Ngoerah General Hospital and Masadini Clinic in Denpasar, Bali, from May to October 2022. Inclusion criteria for the case group were children aged 5 years and under who had been diagnosed with ASD based on DSM-5 criteria[10] by growth and development pediatric consultants. The control group included children aged 5 years and under without ASD. All subjects' parents provided written informed consent. Children with congenital malformations, genetic defects, had consumed zinc supplementation in the previous three months, or whose mothers had consumed valproic acid prenatally or had rubella or cytomegalovirus infections were excluded from the study. Subjects were included by consecutive sampling, the minimum required sample size was 64 for each group. Baseline characteristics were collected by parental interviews. Zinc level (μg/g) was measured in hair specimens by the ICP-MS method[11] at the Udayana University laboratory. Low zinc level was defined as a cut-off of $\leq$ 70 μg/g. Nutritional status was defined using Waterlow criteria.[12] Data were analyzed by Chi-square test for normally distributed variables and Fischer's test as the alternative, using *SPSS version 23* software. Possible confounding factors such as age, gender, and nutritional status were controlled with logistic regression. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Udayana/Prof. I.G.N.G. Ngoerah General Hospital.

Results

Of the 128 children who met the inclusion criteria, 64 subjects were allocated to the case group and 64 to the control group. Baseline characteristics are shown in **Table 1**. The majority of subjects were male (76%) and well nourished (82.3%). The mean maternal age at delivery was 28.82 (SD 3.2) years. Subjects' mean age was 4 (SD 0.8) years and mean hair zinc level was 87.9 (SD 231.1) μg/g.

The zinc cut-off point in children with ASD was defined using a receiver operating characteristic (ROC) curve and evaluation of the area under the curve (AUC). The ROC curve is shown in **Figure 1**. Analysis of the ROC curve revealed 81.4% sensitivity and 80% specificity, with a cut-off point of 33.88 μg/g (**Table 2**). Based on these data, the. risk model of zinc level in ASD is shown in **Table 3**. Children with ASD were 22.19 times more likely to have hair zinc concentration < 33.88 μg/g than children without ASD (OR 22.19; 95%CI 8.02 to 42.90; P< 0.001).

Discussion

The majority of our subjects were male (76%) and 78.1% of our ASD group were male. Similarly,

Table 1. Characteristics of subjects

Characteristics	Case (n=64)	Control (n=64)	Total (N=128)
Gender, n (%)			
Male	50 (78.1)	47 (73.4)	97 (76)
Female	14 (21.9)	17 (26.6)	31 (24)
Nutritional status, n (%)			
Undernourished	9 (14.1)	14 (21.9)	23 (17.7)
Well nourished	55 (85.9)	50 (78.1)	105 (82.3)
Mean age (SD), years	3.93 (0.92)	4.40 (0.54)	4.00 (0.80)
Mean maternal age at delivery (SD), years	28.90 (3.15)	28.40 (3.84)	28.82 (3.20)

Table 2. Sensitivity, specificity, and zinc level cut-off point

Variable	AUC	95%CI	Sensitivity	Specificity	Cut-off point	P value
Zinc level	0.792	0.686 to 0.899	77.1%	75%	33.88	<0.001

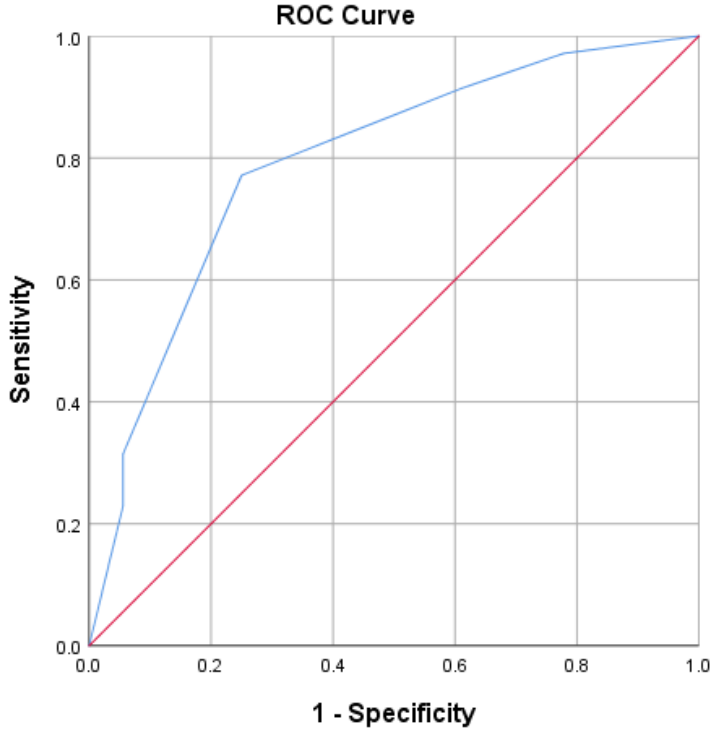

Figure 1. Receiver operating characteristic curve

a previous study in Soeprapto Hospital, Bengkulu, found that most children with ASD were male (70.5%).[13] Autism spectrum disorder occurs 5 times more often in males than females.[14] The role of gender in ASD incidence is still unknown. Further studies are needed to fully understand the biological mechanism and clinical significance of gender difference.

Children with ASD commonly experience gastrointestinal dysfunction, such as diarrhea or constipation, leading to micronutrient malabsorption.[15] A study in *Yayasan Pendidikan Anak Cacat* (YPAC), Surakarta found that 75% of children with ASD were underweight.[16] In contrast, we had more well-nourished subjects (82.3%) overall, and 85.9% of

Table 3. Bivariate analysis of zinc cut-off level of 33.88 µg/g and ASD

Zinc levels	Group		OR	95%CI	P value
	Case (n=64)	Control (n=64)			
Low, n (%)	52 (81.25)	10 (15.62)	22.19	8.02 to 42.90	<0.001
High, n (%)	12 (18.75)	54 (84.38)			

subjects with ASD were well-nourished. This difference might have been due to our comprehensive nutrition management including macro and micronutrient fulfillment that was implemented in the Outpatient Department of Prof. Dr. I. G. N. G. Ngoerah General Hospital and Masadini Clinic.

The mean age of all subjects was 4 (SD 0.8) years and mean age of the case group was 3.93 (SD 0.92) years. A study also reported an age range of 4-5 years in children with autism (80%).[17] Several non-specific environmental factors might have affected the incidence of ASD, namely, maternal age at delivery, which was 28.82 (SD 3.2) years in our study. This result was supported by a previous study which found that mothers aged > 30 years had a 27% higher risk of having children with ASD.[1]

The mean hair zinc level in our study was 87.9 (SD 231.1) μg/g with lower zinc level in children with ASD [31.48 (SD 24.86) μg/g than in children without ASD [107.67 (SD 99.16) μg/g. There was a significant mean difference of zinc level between children with and without ASD, 131.7 (SD 60) μg/g and 184.0 (SD 19) μg/g respectively with P value < 0.05, similar result with a study in 2005.[6] Another study found that children without ASD had lower hair zinc level than children with ASD [(2.9 μg/g vs. 5.4 μg/g, respectively (P=0.0001)].[8] These differing results might have been due to different specimens, as some studies used serum zinc level that is highly variable and affected by daily zinc consumption, while hair zinc level is more stable due to slower metabolism.[5]

Zinc plays an important role in the metabolism of protein, carbohydrate, and fat, collaborating with active forms of numerous metalloenzymes and acting as a neurotransmitter and neuromodulator in the central nervous system. Zinc is used in synaptic transmission, endogenous neuromodulation, nucleic acid metabolism, and brain microtubule growth.[3,4] Previous studies have shown increased incidence of zinc deficiency in children with ASD compared to control.[5-7] Signs and symptoms related to zinc deficiency include neuropsychological signs, learning and memory disturbances, behavioral and emotional problems. In addition, zinc deficiency increases glutamate excitotoxicity, which has been linked to seizure pathogenesis.[18]

In our study, low hair zinc level (<33.88 μg/g) and ASD had a significant association (OR 22.19; 95%CI 8.02 to 42.90; P<0.001). Thus, low hair zinc level was 22.19 times more likely to occur in children with ASD than children without ASD. A study also reported that mean hair zinc level of children with ASD was lower than in children without ASD. They further divided children with ASD into three groups according to functional activity and measured their zinc levels in hair. The low functioning group had significantly lower zinc [150.83 (SD 18.09)] than that of the moderate [192.02 (SD 23.04)] and high functioning [187.44 (SD 22.47)] groups (P<0.01).[5] A study found that 66 out of 78 children with ASD had lower than normal zinc level,[19] another study showed that zinc levels from plasma, red blood cells, or hair were lower in children with ASD compared to their non-ASD counterpart.[6] A study further classified ASD based on its degree of severity with the *Childhood ASD Rating Scale* (CARS) and found a significant correlation between low zinc level and increased fear and anxiety (P=0.022) and poorer verbal communication (P=0.017).[7] A previous study also supported the aforementioned studies, in which 86% of patients with ASD were found to be zinc deficient versus 24% of the non-ASD control group. This finding suggests that zinc deficiency is likely to be common in ASD patients and is a potentially modifiable environmental factor associated with the condition.[20] Zinc supplementation in animal models of ASD had beneficial effects, but clinical trials in human are still needed.[21,22]

The limitations of our study were not fully controlling for biases due to our inability to do matching in some variables. We also did not take into consideration the duration of zinc deficiency, initial onset of ASD, and ASD therapy duration as we compared the two groups. In conclusion, low hair zinc level is more likely to occur in children with ASD than in children without ASD.

Conflict of interest

None declared.

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