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THE ASSOCIATION OF SOCIODEMOGRAPHIC FACTORS AND ZINC STATUS WITH AUTISM RISK IN INDONESIAN TODDLERS: ANALYSIS FROM SEANUTS II DATA

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ABSTRACT

Autism spectrum disorder (ASD) is a multifactorial neurodevelopmental condition shaped by nutritional, biological, and sociodemographic determinants. Zinc is essential for neurodevelopment, yet evidence linking zinc status to autism risk remains inconsistent. This study examined the associations of zinc intake and serum zinc concentration with autism risk in Indonesian toddlers aged 18-36 months and assessed the contribution of sociodemographic factors. Using a cross-sectional secondary analysis of Southeast Asia Nutrition Surveys (SEANUTS) II Indonesia data (2019-2020), 664 children were included for dietary analysis and 103 for serum zinc analysis. Autism risk was screened with the Modified Checklist for Autism in Toddlers (M-CHAT); zinc intake and serum zinc were analysed as continuous variables using binary logistic regression. Neither zinc intake (aOR=1.033; 95% CI: 0.973-1.096) nor serum zinc (aOR=1.005; 95% CI: 0.975-1.036) was independently associated with autism risk. In contrast, urban residence and lower maternal education were consistently associated with higher autism risk across both models (aOR up to 7.876 for urban residence and 10.015 for primary maternal education; all $p<0.05$). These findings suggest that, at the population level, sociodemographic context outweighs zinc status in shaping M-CHAT-based autism risk, underscoring the need to strengthen parenting literacy and to expand equitable, standardized developmental screening, particularly for families with lower maternal education, rather than to rely on micronutrient supplementation alone

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by persistent deficits in social communication and interaction, accompanied by restricted, repetitive patterns of behaviour, interests, or activities. The World

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Health Organization estimates that about 1 in 127 people had autism worldwide in 2021 (World Health Organization, 2025). Reported prevalence nonetheless varies substantially across studies, from 0.22% to 3.60% depending on the diagnostic methodology employed (Wang et al., 2025), with recent systematic reviews reporting pooled global estimates of approximately 0.77% to 1% (Issac et al., 2025; Zeidan et al., 2022). The globally reported upward trend in ASD recognition over recent decades has intensified the urgency to identify modifiable risk and protective factors, particularly in low- and middle-income countries.

In Indonesia, epidemiological data on ASD remain limited and geographically uneven. To date, no national survey has specifically measured ASD prevalence in Indonesia (Ministry of Health Republic of Indonesia, 2023). The largest Modified Checklist for Autism in Toddlers/Revised (M-CHAT/R)-based screening study in Indonesia, conducted by (Aditya et al., 2021) among 965 children aged 16-30 months in Jakarta, identified 15.96% as being at risk for ASD, with 3.52% classified as high-risk. These figures represent one of the few available data points on ASD screening in Indonesia and were obtained from a population already benefiting from urban, facility-based access, a context markedly different from the national reality. In the absence of a national ASD surveillance system, and with standardized screening programs largely unavailable outside urban centres, the true burden of ASD in Indonesian toddlers remains substantially undetected at the community level, particularly in rural areas.

Among nutritional factors, zinc is essential for neurodevelopment, contributing to synaptogenesis, neuroplasticity, and glutamatergic/GABAergic neurotransmission (Ahmadani et al., 2025; Padoan et al., 2024). Deficiency during the 18-36-month developmental window may impair the neural systems underlying the social-communicative behaviours that autism screening tools assess.

Published literature on the zinc-ASD association presents inconsistent findings. A meta-analysis by (Liu et al., 2025), encompassing 4,763 subjects from 25 studies, found significantly lower serum zinc concentrations in children with ASD compared with typically developing controls. Conversely, a systematic review covering 52 studies across 22 countries reported that more than half of the included studies found no significant difference in zinc levels between ASD and control groups (do Nascimento et al., 2023). This inconsistency is likely attributable to heterogeneity in diagnostic criteria, age ranges, zinc measurement methods, and inadequate control for sociodemographic confounders.

Beyond nutritional factors, a growing body of evidence underscores the primacy of sociodemographic determinants in shaping child developmental outcomes. Maternal education is consistently associated with child development outcomes, operating through pathways of parenting knowledge, quality of stimulation, and health-seeking behavior (Black et al., 2017). (Walker et al., 2011), in The Lancet Child Development series, emphasized that environmental and social factors frequently exert greater population-level effects on developmental outcomes than do individual biological variables.

Data from SEANUTS II Indonesia shows that the national prevalence of zinc deficiency remains at 11.9% in children aged 6 months to 12 years (Kekalih et al., 2025). This figure is below the 20% threshold commonly used to indicate a population-level public health concern for low serum zinc concentration according to the International Zinc Nutrition Consultative Group (International Zinc Nutrition Consultative Group, 2007). This indicates that the zinc biological signal may be dampened by a ceiling effect in the distribution of zinc status in the population. This situation allows sociodemographic factors to further dominate the variability of autism risk. Although a few Indonesian studies have examined dietary factors in relation to autism risk, to our knowledge none has simultaneously examined dietary zinc intake, biochemically measured serum zinc, and sociodemographic factors within a single national multicentre dataset. This study therefore aimed to examine the associations between zinc intake and serum zinc concentration (as continuous variables) and autism risk in Indonesian toddlers aged 18-36 months, using secondary data from SEANUTS II Indonesia, and to assess the relative contributions of sociodemographic factors after controlling for confounders.

METHOD

This study employed a cross-sectional design with secondary analysis of the Southeast Asian Nutrition Survey II Indonesia (SEANUTS II ID), conducted from September 2019 to March 2020. SEANUTS II was a multicentre nutritional survey providing data on nutritional status, dietary intake, and biochemical parameters among children aged 0.5-12 years in

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Indonesia. This secondary analysis received ethical approval from the Research Ethics Committee of Dr. Cipto Mangunkusumo Hospital-Faculty of Medicine, Universitas Indonesia (No. ND-510/UN2.F1/ETIK/PPM.00.02/2025).

Of 714 children aged 18-36 months in the SEANUTS II Indonesia dataset, 664 met the inclusion criteria for the primary dietary analysis: age 18-36 months and complete data on M-CHAT score, zinc intake from 24-hour dietary recall, and relevant sociodemographic variables. Children with implausible energy intake, previously diagnosed neurological conditions, or incomplete anthropometric data were excluded. Blood sampling was performed in approximately 15% of the cohort (Kekalih et al., 2025), yielding a biochemical subsample (n=103) in which adequate zinc intake was less frequent than in the full dietary sample (60.2% vs 72.3%); this subsample may therefore not fully represent the broader dietary cohort. Accordingly, analyses of serum zinc and CRP were restricted to the biochemical subsample (n=103), whereas analyses of dietary zinc intake and M-CHAT were conducted in the full dietary sample (n=664).

Autism risk was assessed using the original 23-item Modified Checklist for Autism in Toddlers (M-CHAT), validated in the Indonesian language (Salim et al., 2020; Windiani et al., 2016). Children were classified as at-risk if they met either of two criteria: a total M-CHAT score ≥3, or failure of ≥2 critical items. The M-CHAT is a Level 1 screening tool and does not constitute a clinical diagnosis of ASD.

Zinc intake was assessed by a single 24-hour multiple-pass dietary recall and analysed as a continuous variable (mg/day), since one recall cannot characterize habitual intake. Descriptive categories-adequate (≥3 mg/day, per the 2019 Indonesian RDA for ages 1-3 years) or inadequate (<3 mg/day)-were used only for subject characterization in Table 1. Serum zinc was measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and analysed as continuous (µg/dL), with the IZiNCG cut-off of ≥65 µg/dL applied only descriptively in Table 1. Serum C-reactive protein (CRP) was measured by immunoturbidimetric-CMIA (normal ≤5 mg/L; elevated >5 mg/L) and assessed as a potential confounder of the serum zinc-autism association, because systemic inflammation transiently suppresses circulating zinc (Karakochuk et al., 2017). Dietary recall and blood sampling were not performed on the same day in the SEANUTS II protocol.

The sociodemographic factors analysed were area of residence, maternal education, family income, and caregiver respondent type, as these can influence parenting patterns, access to health services, nutritional intake, and child developmental risk. Area of residence was classified as urban or rural. Maternal education was grouped into primary (elementary/junior high or equivalent), secondary (high school or equivalent), and higher (diploma/bachelor's degree). Family income was dichotomized relative to the Provincial Minimum Wage (UMP; national average Rp. 3,507,690). Caregiver respondent type was classified as nuclear-family caregiver (biological parents or siblings in the same household, with consistent daily observation of the child) or extended/non-family caregiver (grandparents, aunts, uncles, housemaids, or other daily caregivers).

Additional covariates included in the bivariate analysis were sex, height-for-age Z-score (HAZ), and history of preterm birth, treated as potential confounders because they may independently influence neurobehavioral development. Preterm birth was defined as gestational age <37 completed weeks, and HAZ was calculated using the WHO Child Growth Standards.

Analyses used IBM SPSS Statistics version 20. Continuous data are presented as median (range) and categorical data as frequency and percentage. Normality was assessed with the Kolmogorov-Smirnov test; zinc intake, serum zinc, and CRP were non-normally distributed and were compared using the Mann-Whitney U test, while categorical associations with autism risk used Pearson's Chi-square or Fisher's exact test. Multivariate analysis used binary logistic regression (enter method), with zinc intake (mg/day) and serum zinc (µg/dL) entered as continuous exposures in two separate models. Candidate confounders were entered into each model when their bivariate association with autism risk reached $p<0.20$; the primary exposures (zinc intake and serum zinc) were retained in all models regardless of their bivariate p value. CRP was additionally included a priori in Model 2 as a biological confounder of serum zinc, given that systemic inflammation suppresses circulating zinc. The final models therefore present the variables of interest together with retained confounders, whether statistically significant or included for confounding control. Model 1 (full dietary sample, N=664) was adjusted for area of residence, maternal education, sex, and caregiver respondent type; Model 2 (biochemical subsample, N=103) was adjusted for CRP, area of residence, and maternal education. To limit overfitting in the smaller subsample, Model 2 was kept parsimonious. Statistical significance was set at $p<0.05$ (two-tailed).

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RESULT AND DISCUSSION

A total of 664 children aged 18-36 months with complete dietary and M-CHAT data were included in the primary analysis. The median age was 23.9 months (range: 18.0-35.97), with approximately equal proportions of female (49.8%) and male (50.2%) children. Slightly more than half of all children resided in rural areas (50.5%). Primary maternal education was the most prevalent category (45.8%), followed by secondary (39.5%) and higher education (14.8%). Most households had income below the Provincial Minimum Wage (70.3%). The caregiver respondent was a nuclear-family caregiver in 83.3% of cases; the remaining 16.7% were extended/non-family caregivers, including grandparents, aunts, uncles, or housemaids, reflecting diverse caregiving arrangements in Indonesian families. Stunting was present in 28.6% of children, and 5.3% had a history of preterm birth. The median zinc intake was 4.10 mg/day (range: 0.95-17.55), with 72.3% classified as having adequate intake (≥3 mg/day per the 2019 Indonesian RDA). The median M-CHAT score was 3 (range: 0-13); 57.4% of children were classified as at-risk by M-CHAT criteria.

Of the 664 children, 103 had complete biochemical data (serum zinc and CRP) and constituted the biochemical subsample for secondary analysis. In this subsample, the median serum zinc was 75 µg/dL (range: 55-150), with 83.5% having adequate serum zinc levels (≥65 µg/dL); only 17 children (16.5%) had inadequate serum zinc. Median CRP was 0.60 mg/L, and 18.4% (n=19) had elevated CRP (>5 mg/L). The median M-CHAT score was 3 (range: 0-8), and 62.1% of children were classified as at risk for autism.

Table 1. Subject Characteristics of Dietary Sample and Biochemical Subsample

Characteristic	n (%)
A. Dietary Sample (N=664)	
Age (months)	23.9 (18.0-35.97)
Sex	
Female	331 (49.8%)
Male	333 (50.2%)
Area of Residence	
Rural	335 (50.5%)
Urban	329 (49.5%)
Maternal Education	
Higher	98 (14.8%)
Secondary	262 (39.5%)
Primary	304 (45.8%)
Household Income	
≥ Provincial Minimum Wage	197 (29.7%)
< Provincial Minimum Wage	467 (70.3%)
Nutritional Status: Stunting (HAZ)	190 (28.6%)
History of Preterm Birth	35 (5.3%)
Caregiver Type	
Nuclear-family caregiver	553 (83.3%)
Extended/non-family caregiver[a]	111 (16.7%)
M-CHAT Score	3 (0-13)

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Characteristic	n (%)
Normal by M-CHAT criteria	283 (42.6%)
At-risk by M-CHAT criteria	381 (57.4%)
Zinc Intake (mg/day)	4.10 (0.95-17.55)
Adequate (≥3 mg/day)	480 (72.3%)
Inadequate (<3 mg/day)	184 (27.7%)
B. Biochemical Subsample (N=103)	
Serum Zinc (µg/dL) (N=103)	75 (55-150)
Adequate (≥65 µg/dL)	86 (83.5%)
Inadequate (<65 µg/dL)	17 (16.5%)
CRP (mg/L) (N=103)	0.60 (0.10-43.0)
Normal (≤5 mg/L)	84 (81.6%)
Elevated (>5 mg/L)	19 (18.4%)

HAZ = height-for-age Z-score; CRP = C-reactive protein. Zinc intake and serum zinc categorizations are presented for descriptive purposes only and were not used in bivariate or multivariate analyses. Adequate serum zinc defined as ≥65 µg/dL per IZiNCG criteria.
[a]Extended/non-family caregivers include grandparents, aunts, uncles, and housemaids
Source: Authors' analysis of SEANUTS II Indonesia secondary data (2019-2020)

Bivariate analysis was conducted separately for the full dietary sample (N=664) and the biochemical subsample (N=103). In the full dietary sample, zinc intake was not significantly different between the normal and at-risk groups [4.23 mg/day (0.98-14.59) vs. 4.05 mg/day (0.95-17.55); p=0.966]. Age also showed no significant difference between groups [23.50 months (18.00-35.87) vs. 24.40 months (18.00-35.97); p=0.419]. Among sociodemographic variables, area of residence and maternal education were significantly associated with autism risk. A higher proportion of children in the at-risk group lived in urban areas compared with the normal group (54.9% vs. 42.4%; p=0.002). Maternal education also showed a significant association with M-CHAT-based autism risk (p=0.006). The at-risk group had a lower proportion of mothers with higher education compared with the normal group (11.0% vs. 19.8%) and a higher proportion of mothers with primary education (48.8% vs. 41.7%).

Other variables were not significantly associated with M-CHAT-based autism risk, including sex (p=0.114), household income (p=0.496), stunting status based on HAZ (p=0.865), history of preterm birth (p=0.279), and caregiver respondent type (p=0.106). In the biochemical subsample, serum zinc concentration was not significantly different between the normal and at-risk groups [75.00 µg/dL (56-131) vs. 75.00 µg/dL (55-150); p=0.713]. CRP levels also did not differ significantly between groups [0.50 mg/L (0.10-43.00) vs. 0.60 mg/L (0.10-34.60); p=0.782]. Overall, these findings indicate that M-CHAT-based autism risk was significantly associated with area of residence and maternal education, but not with zinc intake, serum zinc concentration, CRP, or other clinical and respondent-related characteristics.

Table 2. Bivariate Association between Subject Characteristics and M-CHAT-based Autism Risk

Variable	Normal n (%) / Median (min-max)	At-Risk n (%) / Median (min-max)	p value
A. Dietary sample (N=664)	**n=283**	**n=381**	
Zinc intake, mg/day	4.23 (0.98-14.59)	4.05 (0.95-17.55)	0.966[mw]

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Variable	Normal n (%) / Median (min-max)	At-Risk n (%) / Median (min-max)	p value
A. Dietary sample (N=664)	**n=283**	**n=381**	
Age, months	23.50 (18.00-35.87)	24.40 (18.00-35.97)	0.419 [mw]
Sex			**0.114[cs]**
Female	131 (46.3%)	200 (52.5%)	
Male	152 (53.7%)	181 (47.5%)	
Area of residence			**0.002[cs]**
Rural	163 (57.6%)	172 (45.1%)	
Urban	120 (42.4%)	209 (54.9%)	
Maternal education			**0.006[cs]**
Higher	56 (19.8%)	42 (11.0%)	
Secondary	109 (38.5%)	153 (40.2%)	
Primary	118 (41.7%)	186 (48.8%)	
Household income			**0.496[cs]**
≥ Provincial Minimum Wage	80 (28.3%)	117 (30.7%)	
< Provincial Minimum Wage	203 (71.7%)	264 (69.3%)	
Nutritional status, HAZ			**0.865[cs]**
Normal	203 (71.7%)	271 (71.1%)	
Stunted	80 (28.3%)	110 (28.9%)	
History of preterm birth			**0.279[cs]**
No	265 (93.6%)	364 (95.5%)	
Yes	18 (6.4%)	17 (4.5%)	
Caregiver respondent type			**0.106[cs]**
Nuclear-family caregiver	228 (80.6%)	325 (85.3%)	
Extended/non-family caregiver	55 (19.4%)	56 (14.7%)	
B. Biochemical subsample (N=103)	**n=39**	**n=64**	
Serum zinc, µg/dL	75.00 (56-131)	75.00 (55-150)	0.713[mw]
CRP, mg/L	0.50 (0.10-43.00)	0.60 (0.10-34.60)	0.782[mw]
Area of residence			**<0.001[cs]**
Rural	31 (79.5%)	29 (45.3%)	
Urban	8 (20.5%)	35 (54.7%)	
Maternal education			**0.042[cs]**
Higher	11 (28.2%)	6 (9.4%)	
Secondary	14 (35.9%)	27 (42.2%)	
Primary	14 (35.9%)	31 (48.4%)	

[mw] = Mann-Whitney U test; [cs] = Chi-square/Fisher's exact test; CRP = C-reactive protein; HAZ = height-for-age Z-score; Bold = p<0.05

Data are presented as n (%) for categorical variables and median (minimum-maximum) for continuous variables. Percentages are column percentages. Mann-Whitney U test was used for continuous variables. Pearson's Chi-square or Fisher's exact test was used for categorical variables, as appropriate. Dietary analyses were performed in the full dietary sample (N=664), while serum zinc and CRP analyses were performed in the biochemical subsample (N=103).

Source: Authors' analysis of SEANUTS II Indonesia secondary data (2019-2020)

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Multivariate analysis was conducted using two binary logistic regression models. Model 1 used the full dietary sample (N=664) to examine the association between zinc intake and M-CHAT-based autism risk after adjustment for area of residence, maternal education, sex, and caregiver respondent type. Model 2 used the biochemical subsample (N=103) to examine the association between serum zinc concentration and M-CHAT-based autism risk after adjustment for CRP, area of residence, and maternal education.

In Model 1, zinc intake was not independently associated with M-CHAT-based autism risk (aOR=1.033; 95% CI: 0.973-1.096; p=0.287). Urban residence was associated with higher odds of autism risk compared with rural residence (aOR=1.699; 95% CI: 1.228-2.351; p=0.001). Lower maternal education also showed significant associations. Compared with higher maternal education, secondary education (aOR=1.880; 95% CI: 1.163-3.037; p=0.010) and primary education (aOR=2.379; 95% CI: 1.472-3.845; p<0.001) were associated with higher odds of autism risk. Sex and caregiver respondent type were not significant.

In Model 2, serum zinc concentration was not independently associated with autism risk (aOR=1.005; 95% CI: 0.975-1.036; p=0.743). CRP was also not significant (aOR=0.965; 95% CI: 0.912-1.020; p=0.203). Urban residence remained significantly associated with autism risk (aOR=7.876; 95% CI: 2.529-24.525; p<0.001). Secondary maternal education (aOR=6.231; 95% CI: 1.523-25.497; p=0.011) and primary maternal education (aOR=10.015; 95% CI: 2.319-43.247; p=0.002) also remained significant.

Both models were statistically significant and showed acceptable fit. Model 1 had a Nagelkerke R^2 of 0.056 and a Hosmer-Lemeshow p-value of 0.755, while Model 2 had a Nagelkerke R^2 of 0.287 and a Hosmer-Lemeshow p-value of 0.187. Full results are presented in Table 3.

Table 3. Multivariate Binary Logistic Regression Analysis of Factors Associated with M-CHAT-based Autism Risk

Variable	β	Crude OR (95% CI)	aOR (95% CI)	p value
Model 1: Dietary zinc model (N=664)				
Zinc intake, per 1 mg/day increase	0.032	1.031 (0.975-1.090)	1.033 (0.973-1.096)	0.287
Residence: Rural	-	Ref	Ref	-
Residence: Urban	0.530	1.651 (1.210-2.251)	1.699 (1.228-2.351)	**0.001**
Maternal education: Higher	-	Ref	Ref	-
Maternal education: Secondary	0.631	1.872 (1.170-2.993)	1.880 (1.163-3.037)	**0.010**
Maternal education: Primary	0.867	2.102 (1.324-3.335)	2.379 (1.472-3.845)	**<0.001**
Sex: Female	-	Ref	Ref	-
Sex: Male	-0.251	0.780 (0.573-1.062)	0.778 (0.567-1.067)	0.120
Caregiver respondent type: Nuclear-family caregiver	-	Ref	Ref	-
Caregiver respondent type: Extended/non-family caregiver	-0.326	0.714 (0.475-1.075)	0.722 (0.476-1.095)	0.125
Model 2: Serum zinc model (N=103)				
Serum zinc, per 1 µg/dL increase	0.005	0.994 (0.970-1.019)	1.005 (0.975-1.036)	0.743
CRP, per 1 mg/L increase	-0.036	0.979 (0.933-1.028)	0.965 (0.912-1.020)	0.203
Residence: Rural	-	Ref	Ref	-
Residence: Urban	2.064	4.677 (1.864-11.735)	7.876 (2.529-24.525)	**<0.001**
Maternal education: Higher	-	Ref	Ref	-
Maternal education: Secondary	1.830	3.536 (1.080-11.574)	6.231 (1.523-25.497)	**0.011**

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Variable	β	Crude OR (95% CI)	aOR (95% CI)	p value
Maternal education: Primary	2.304	4.060 (1.250-13.185)	10.015 (2.319-43.247)	**0.002**

Outcome variable: M-CHAT-based autism risk, coded as 0 = normal and 1 = at-risk; Crude OR = crude odds ratio; aOR = adjusted odds ratio; β = logistic regression coefficient; Ref = Reference category; Bold = p<0.05; CI = confidence interval; CRP = C-reactive protein. Model 1 was adjusted for area of residence, maternal education, sex, and caregiver respondent type. Model 2 was adjusted for CRP, area of residence, and maternal education.
Source: Authors' analysis of SEANUTS II Indonesia secondary data (2019-2020)

This study found no statistically significant association between zinc status and M-CHAT-based autism risk in Indonesian toddlers; the null finding held for both dietary zinc intake (N=664) and serum zinc concentration (N=103) after adjustment. In contrast, area of residence and maternal education showed the most consistent associations with autism risk across bivariate and multivariate analyses (Shahid Khan et al., 2024), with urban residence and lower maternal education linked to higher odds of screening at-risk.

The absence of an independent zinc-autism association should be interpreted within the population's nutritional profile: median zinc intake (4.10 mg/day) and serum zinc (75 µg/dL, adequate in 83.5%) were relatively high, so few children clustered at the low end of zinc status where an association might be detectable. This is consistent with findings that more than half of studies found no significant difference in zinc levels between children with ASD and controls (do Nascimento et al., 2023). However, it contrasts with Liu et al. (2025), who reported lower serum zinc levels in children with ASD. This discrepancy likely reflects heterogeneity in age, diagnostic criteria, inflammation control, dietary assessment, and population-level zinc deficiency. Supporting a bioavailability interpretation, (Pattisahusiwa, 2026), using the same SEANUTS II dataset for children aged 6-23 months, found the median phytate-to-zinc ratio rose nearly fourfold with age (2.31 to 9.61) and correlated negatively with linear growth (LAZ; r=−0.079; p=0.034), suggesting that zinc intake alone may not reflect functional zinc status in cereal-based, high-phytate diets.

Several methodological factors may also explain the null zinc findings. First, zinc intake was assessed using a single 24-hour dietary recall, which captures only short-term intake and may not represent habitual zinc exposure. Second, serum zinc reflects recent physiological status and is affected by infection, inflammation, time of blood collection, and recent dietary intake. In this study, CRP was included in the serum zinc model because systemic inflammation can reduce circulating zinc through acute-phase redistribution. CRP itself was not independently associated with autism risk, and adjustment for CRP did not change the main finding that serum zinc was not a significant associated with M-CHAT-based autism risk. Third, dietary recall and blood sampling were not conducted on the same day in the SEANUTS II protocol, limiting direct interpretation of the intake-biomarker relationship. Therefore, the null finding should not be interpreted as evidence that zinc has no role in neurodevelopment, but rather that zinc status did not independently predict M-CHAT-based autism risk in this specific dataset.

Area of residence was consistently associated with M-CHAT-based autism risk: urban residence carried 1.70-fold higher odds in the dietary model and 7.88-fold in the biochemical model, though the latter rests on a smaller sample with a wider confidence interval. Several mechanisms may contribute. M-CHAT is caregiver-reported, and urban caregivers may have greater exposure to developmental information, increasing recognition and reporting of atypical behaviours (De Jonge et al., 2024). Urban environments may also entail different developmental exposures (e.g., higher screen time, reduced outdoor play and multi-generational interaction, air pollution), and SEANUTS II reported higher zinc deficiency among urban than rural children aged 1-3.9 years. These remain hypotheses, as the study did not measure screen time, pollutants, stimulation quality, or family structure.

In Model 2, adjusted odds ratios for urban residence and lower maternal education were substantially larger than crude estimates (urban: 4.677→7.876; secondary education: 3.536→6.231; primary education: 4.060→10.015), a pattern consistent with negative confounding by CRP and serum zinc, whose distribution appears to have partially masked the sociodemographic effects before adjustment. Model 1, by contrast, showed high crude-to-adjusted stability, consistent with its larger sample (N=664). The amplified Model 2 estimates should be read cautiously given the small subsample (N=103) and wide confidence intervals.

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The high at-risk proportions (57.4% dietary; 62.1% biochemical) represent screening positivity, not clinical ASD prevalence. Because the SEANUTS II protocol used the original M-CHAT without the M-CHAT-R/F structured follow-up, false positives are more likely, particularly in community samples where the tool may capture broader developmental, language, and caregiver-report variation rather than confirmed ASD. Future Indonesian studies should apply M-CHAT-R/F with DSM-5-based clinical confirmation.

Maternal education was independently associated with autism risk in both models, with lower education (secondary or primary) carrying higher odds than higher education. This graded pattern is consistent with maternal education acting as a social determinant of early development-shaping knowledge of milestones, home stimulation, health-seeking behaviour, and access to screening and referral-and aligns with prior evidence (Black et al., 2017; Rezaeizadeh et al., 2024; Walker et al., 2011).

Caregiver respondent type was not independently associated with M-CHAT-based autism risk, despite being included because M-CHAT relies on caregiver report. The variable is limited, however, as it does not capture caregiving quality, time with the child, or family structure; in Indonesia, extended-family caregiving may shape child social interaction and observation patterns that the available SEANUTS II variables could not fully reflect.

Two unmeasured factors warrant future attention. First, developmental stimulation (verbal interaction, responsive play, reading, caregiver involvement) is directly relevant to social-communication outcomes but was not captured; future studies should use validated tools such as the Infant-Toddler Home Observation for Measurement of the Environment (IT-HOME) or the Parenting Interactions with Children: Checklist of Observations Linked to Outcomes (PICCOLO). Second, family structure and caregiving ecology may differ between nuclear and extended households in caregiver availability and social interaction and should be recorded as potential confounders.

This study has several strengths and limitations. This study is, to our knowledge, the first in Indonesia to examine zinc status and M-CHAT-based autism risk in toddlers aged 18-36 months while integrating dietary zinc intake, ICP-MS-measured serum zinc, CRP, and sociodemographic determinants in one framework. ICP-MS provides precise serum zinc measurement that would be costly to reproduce de novo, and the SEANUTS II data span 21 districts across Java and Sumatra, offering geographic diversity rarely available in Indonesian toddler datasets.

Several limitations apply. The cross-sectional design precludes causal inference. Autism risk was based on the original M-CHAT without structured follow-up, so it reflects screening positivity rather than clinical ASD. Zinc assessment was constrained by a single 24-hour recall, a small biochemical subsample, and non-concurrent dietary and blood sampling. These constraints are inherent to the SEANUTS II design; because no alternative Indonesian dataset combines M-CHAT, dietary zinc, ICP-MS serum zinc, CRP, and sociodemographic data for this age group, the findings are best interpreted as exploratory evidence to guide future primary studies.

CONCLUSION

This study found that zinc intake and serum zinc concentration, analysed as continuous variables, were not independently associated with M-CHAT-based autism risk among Indonesian toddlers aged 18-36 months. In contrast, area of residence and maternal education showed consistent associations with M-CHAT-based autism risk. Urban residence was associated with higher odds of autism risk in both the dietary model and the biochemical model. Lower maternal education also showed a graded association, with higher odds observed among children whose mothers had secondary or primary education compared with higher education.

From a public health perspective, these findings support two program priorities: strengthening caregiver education on early child development, particularly in families with lower maternal education, and expanding equitable access to standardized developmental screening and referral pathways through community-based child health services. Urban strategies may prioritize scheduled M-CHAT-R/F screening, caregiver education on screen-time practices, and structured developmental surveillance. Rural strategies may focus on community health worker capacity-building, simplified referral pathways, and integration of developmental screening into *Posyandu* services.

Future research should employ longitudinal designs with repeated dietary assessments (multiple 24-hour recalls or semi-quantitative food frequency questionnaires), zinc bioavailability estimation, validated instruments for measuring

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developmental stimulation (Infant-Toddler Home Observation for Measurement of the Environment/IT-HOME or Parenting Interactions with Children: Checklist of Observations Linked to Outcomes/PICCOLO), and clinical DSM-5-based confirmation of M-CHAT-positive cases to accurately estimate ASD prevalence rather than screening positivity.

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