https://doi.org/10.7555/JBR.37.20230295
Original article

Title: Buccal DNA global methylation and cognitive performance in stunted children under 5 years of age

Running title: DNA methylation and cognitive in stunted children

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Received: 02 December 2023; Revised: 05 August 2024; Accepted: 09 August 2024;
CLC number: R179, Document code: A
The authors reported no conflict of interests.

Abstract

The prevalence of stunting in Indonesian children under five years of age is about 20%. Chronic maternal malnutrition contributes to the risk of stunting by affecting global DNA methylation. In the present study, we aimed to evaluate the levels of 5-methyl-cytosine (5mC), as a surrogate marker of global DNA methylation, in buccal swabs and its potential association with risk of stunting and cognitive performance. The levels of 5mC were measured using the enzyme-linked immunosorbent assay. The Wechsler Preschool and Primary Scale of Intelligence was used to measure cognitive functions. Buccal swab DNA samples and anthropometric data were collected from a total of 231 children aged zero to five years. In this cross-sectional cohort, the prevalence of stunting was 37% in 138 children aged zero to two years and 30% in 93 children aged > two years. The univariable analysis revealed that the levels of 5mC in buccal swab DNA were significantly lower in severely stunted children (median, 2.84; interquartile range [IQR], 2.39–4.62; *P*-value, 0.0314) and in children of a younger age (median, 2.81; IQR 2.53–4.62, *P*-value, 0.0001) than in normal (median, 3.75; IQR, 2.80–4.74) and older children (median, 4.01, IQR, 3.39–4.87), respectively. We also found that the average cognitive scores tended to be low in boys and stunted children, although the differences were not statistically significant. Furthermore, levels of 5mC found in buccal and mouthwash DNA were not associated with cognitive scores.

Keywords: global DNA methylation, 5mC, ELISA, stunting, cognitive

Introduction

Stunting, defined by the World Health Organization as having a height-for-age Z score (HAZ) less than –2 standard deviation[1], is a public health burden, because it reduces the quality of life in adulthood, such as contracting non-communicable diseases [2] and risk of low cognitive performance[3]. A national nutrition survey conducted by the Ministry of Health Directorate of the Republic of Indonesia in 2017 reported a prevalence of stunting of approximately 20.1 % among children below two years of age[4]. Regional clusters in Indonesia have a higher prevalence of stunting than the national average; for example, in the Pandeglang district in western Java, the prevalence of stunting is about 30%[5].

Chronic malnutrition is one of the established risk factors for stunting, particularly maternal malnutrition during the first 1000 days after the gestational period[6]. During this period, totipotency of embryonic stem cells is critical for healthy development, which is achieved by erasing parental 5-methyl-cytosine (5mC) of the fetal genome[7]. 5mC is then reintroduced into the fetal genome to induce tissue growth and differentiation. Therefore, the reintroduction of 5mC may be affected by inadequate nutritional intake of pregnant mothers[8]. During embryonic development, the 1-carbon metabolism pathway plays a crucial role in providing methyl groups (such as folate, betaine, methionine, and serine) and cofactors (*e.g.*, vitamins B2, B6, and B12) for DNA methylation[9]. For example, maternal diet may affect DNA methylation of the infants[10] and the lack of methyl groups or cofactors in the maternal diet led to DNA hypomethylation in Jamaican children[11]. On the other hand, an epigenetic study in slum areas of Bangladesh, focusing on children aged two to three years, demonstrated a significant association of lower protein and calorie intake on high global DNA methylation. However, whether malnutrition manifested in stunting is associated with low or high global DNA methylation requires further study.

Stunting may lead to poor a neural development affecting cognitive performance during school years and later in adulthood[2]. More importantly, a persistent early-onset stunting leads to low cognitive scores [3]. Poor cognitive functions may be the result of defects in neural development, as observed in animal models of malnutrition[12].

Interventions to prevent stunting have faced many challenges, including lack of compliance of participants[13–14], the absence of relevant biomarkers to address specific micronutrient deficiencies[15], and non-standardized anthropometric measurements[16] that may affect the

precision of the prevalence of stunting. Recently, buccal cells and neurons have been shown to share similar global DNA methylation patterns[17]. Therefore, buccal cells that are readily accessible in children may serve as surrogate specimens for cognitive studies. In the present study, we aimed to assess the association of 5mC levels in buccal DNA collected by a less invasive method with stunting or cognitive performance.

Subjects and methods

Study population and design

The present study was a cross-sectional investigation that included children aged zero to five years (n=231), who attended local community health facilities in three villages (Kadumanueh, Kadubelang, and Medong) in Pandeglang district, Banten province, Indonesia. The prevalence of stunting in Pandeglang, especially in Kadumaneuh, has been estimated at around 30% according to previous studies[5,18]. The sample size needed for the analysis was not calculated, because no references in the literature had described global DNA methylation in buccal specimens in children with stunting. However, cross-sectional non-stunting studies using buccal DNA as a source of global DNA methylation in children had recruited between 48 to 73 children[19–20]. Considering the budget, we conducted a consecutive sampling of children who attended community healthcare facilities in the villages on 10 September 2022, from 9 a.m. to 12 p.m., to collect their buccal cells and anthropometric measurements. There were four healthcare facilities in each village amounting to a total of 12 recruitment sites. Cognitive measurements were performed on children aged four years or older within one month after recruitment. A diagram of subject recruitment, inclusion criteria, and analyses is shown in ***Fig. 1***.

Buccal DNA samples were collected from all children (n = 231), consisting of 138 children aged two years old or younger and 93 children older than two years old. In addition, children older than two years old (n = 93) were offered to participate in DNA mouthwash collection. Successful mouthwash collection was obtained in 80 children. Since stunting may also affect cognitive function, a subset of these 80 children, *i.e.*, children aged four years and older (n = 55), participated and completed cognitive evaluation. After a detailed explanation of the study, we obtained the informed consent from their parents or guardians, and the present study was conducted following the Helsinki declaration. This study was approved by the YARSI Ethics Committee No. 238/KEP-LPUY/VIII/2022.

Anthropometric status

We measured the height to the nearest 0.1 cm in the standing position. The weight was measured to the nearest 0.05 kg. The height/length of the children was measured directly by physical examination. The height of the child was measured in centimeters according to the standards of the Ministry of Health of the Republic of Indonesia as previously described[5]. The measuring instrument used was an RGZ-20A microtoise Gea baby scale, and baby GEA WBC stature gauge. The HAZ for children was calculated using the WHO growth standard. Children with HAZ (height-for-age Z score) < −2 and < −3 were considered stunted and severely stunted, respectively. The non-stunted control was defined as HAZ of > −1.

DNA collection and extraction

Buccal cheek swabs were collected from all participants using commercial buccal DNA isolation kits. Parents were asked not to give meals to their children 30 min before sample collections. To collect the buccal cells, 41 trained medical students rubbed a sterile swab head against the inside cheeks of the child for 20 rounds on each cheek. In addition, children aged four to five years old were also asked to take about 2.5 mL of mouthwash liquid provided by the kit solution and spit it into the preservative solution[21]. The samples were stored in a sterile tube at room temperature and transported to the YARSI University molecular testing laboratory. DNA was obtained using an automated DNA extraction kit using magnetic bead technology (Maxwell Promega, USA). The DNA concentration was quantified using spectrophotometry.

Epigenetic measurement of global DNA methylation

Global DNA methylation of child buccal cheek swabs was analyzed using an (indirect) ELISA-based commercial kit (Methyl Flash Methylated DNA 5mC Quantification Kit (Colorimetric), EpiGentek as previously described[22]. Briefly, 100 ng of input DNA was bound to the wells and methylated DNA was detected using antibodies to 5mC and then colorimetrically quantified by reading absorbance at 405 nm. The standard curve was generated using the absorbance values of seven standards made by mixing positive and negative controls provided by the kit. The final standard methylation concentrations were 0%, 5%, 10%, 25%, 50%, 75%, and 100%, respectively. The 5mC levels for unknown DNA samples were calculated

as follows: %5mC = e (Absorbance y-intercept)/slope. The absorbance was measured at 405 nm using an ELISA plate reader (Tecan Infinite M200 Pro, Switzerland).

Cognitive evaluation

Child cognitive function was evaluated using the Wechsler Preschool and Primary Scale of Intelligence, which is suitable for children aged four to 7.3 years as described previously[23]. Ten trained psychologist assistants who has degree of Bachelor of Psychology conducted the assessment, which was closely supervised by a licensed Psychologist (OIR). The assessment was administered in each child's house to minimize unfamiliar surroundings that may distract children's attention during the test. The administration of the test took 60–70 min. The test was paused when the child looked tired or bored. During the break, the assistant offered the child to have a snack or drink and talked about the toy or game they favored. The break usually took five min before the assistant asked the participant to continue the test. When the child refused to continue the test, the assistant stopped the assessment and came back in the next day.

Cognitive function was measured using the Indonesian version of the Wechsler of Preschool and Primary Scale of Intelligence (WPPSI) published by the Faculty of Psychology Universitas Indonesia[23]. The WPPSI consists of two scales, the Verbal Scale and Performance Scale. The Verbal Scale was measured by five subtests: information, vocabulary, comprehension, similarities, and arithmetic. The Performance Scale was measured by other five subtests, which are Animal House, Picture Completion, Mazes, Geometric Design, and Block Design. The raw score of each subtest was standardized according to age group before summing the score for each scale and converted into Verbal intelligence quotient (VIQ) and Performance IQ (PIQ). The standard scores of the Verbal and Performance Scale were summed and converted into Full Scale IQ (FIQ)[23].

Statistical analysis

We used the Chi-square test to compare the proportion of normal, stunted, and severely stunted children in different independent variables such as sex, age, and villages. Since the values of 5mC levels were not normally distributed, we compared the median of 5mC levels in multiple groups of HAZ status (*i.e.*, normal, stunted, and severely stunted children) and age (zero to 24 months, 24 to 48 months and older than 48 months) using Kruskal-Wallis rank test. When

the Kruskal-Wallis test showed statistical significance, post hoc Dunn-Bonferroni tests were calculated to reveal differences among different subgroups (HAZ status and age groups). Mann-Whitney test was used to compare the median values of 5mC levels between boys and girls. Binary logistic regression was used to test numerical data (5mC levels) as a predictor of binary stunting outcomes in different age groups. To determine the relationship between global DNA methylation and cognitive function, we used linear regression model as described previously[22]. A two-sided *P*-value < 0.05 was considered statistically significant. All statistical analyses were performed using Statplus for Mac statistical software (AnalystSoft Inc., Walnut, CA, USA), GraphPad Prism version 10.0.0 for Windows (GraphPad Software, Boston, MA, USA, www.graphpad.com), and DATAtab: Online Statistics Calculator (DATAtab e.U. Graz, Austria, https://datatab.net).

Results

Demographic of participants

Children ranged in age from two to 73 months, with a mean age of 28.4 months (SD = 20.1 months) and a median age of 19 months (IQR: 12–49 months). As shown in ***Table 1***, the prevalence of stunting ($-3 \leq HAZ < -2$) and severe stunting ($HAZ < -3$) in this cohort was 21% and 13% out of 231 subjects, respectively. Moreover, prevalence of stunting was 37% in children aged zero to two years and 30% in children aged more than 2 years. Approximately 57% and 60% of this cohort were boys and children aged zero to two years old, respectively. Of the three villages, Kadumaneuh had the highest prevalence of severe stunting (50%). The average concentration of buccal DNA and the corresponding 5mC levels were 28.3 ng/μL and 4.06%, respectively.

Stunting prevalence among participants

As shown in ***Table 2***, stunting outcomes were not significantly associated with age, sex, or the villages in which children had lived (*P*-values > 0.05).

Association of the levels of 5mC in buccal swab DNA with age, sex, and HAZ

As shown in ***Table 3***, the levels of 5mC were significantly associated with age, sex, and HAZ status. Specifically, normal children had significantly higher 5mC levels than severely

stunted children, and the youngest age group (zero to 24 months) had the lowest levels of 5mC compared with older age groups.

The distribution of 5mC levels by age and HAZ status is shown in ***Fig. 2***. Because the 5mC levels were not normally distributed, the Spearman correlation analysis was used to examine the correlations between 5mC levels and age in different HAZ statuses. As shown in ***Table 4***, a significant correlation was found between age and 5mC levels in stunted children ($-3 \leq HAZ < -2$), but not in normal ($HAZ \geq -2$) or severely stunted ($HAZ < -3$) children.

Although the 5mC levels were lower in severely stunted children, we tested whether this association remained with adjustment for age and sex in this cohort ($n = 231$). The results of multivariate logistic regression analysis showed that the outcome of stunting (including both stunted and severely stunted children with an $HAZ < -2$) was not significantly associated with age (coefficient, 0.0036; 95% CI, −0.010–0.0177), 5mC levels (coefficient, 0.099; 95% CI, −0.039–0.278), and sex (coefficient, 0.252; 95% CI, −0.304–0.808).

Association of 5mC levels in mouthwash DNA with HAZ and sex

Mouthwash DNA was another source of non-invasive DNA collection, which did not require swabbing tools. Out of 98 children aged more than two years, mouthwash DNA was not successfully extracted from 80 children. Therefore, there were 80 children with paired DNA collected from buccal swabs and mouthwash. The 5mC levels in swab DNA (median, 4.01; IQR, 3.4–4.7) were significantly higher than mouthwash DNA (median, 3.69; IQR, 3.27–4.20) ($P = 0.018$ by Wilcoxon matched-pairs signed rank test). There was no significant difference in 5mC levels in mouthwash DNA in children with different HAZ and sex (***Table 5***).

Association of 5mC levels, stunting, and cognitive performance

Of the 80 children, 55 aged four years and over completed the cognitive assessment. For the three cognitive scores (PIQ, VIQ, and FIQ), the differences in performance attributed to sex and HAZ were not significant (***Table 6***). Of note, children who lived in Kadumaneh village had significantly lower VIQ and FIQ scores than those living in the other two villages.

To assess different variables that might influence performance IQ scores, we performed multivariable regression analysis, and the results are shown in ***Table 7***. None of the variables had a significant association with cognitive performance.

Discussion

In the present study, a sufficiently high concentration of DNA that is suitable for epigenetic analysis was collected from buccal swabs and mouthwash. Both methods were non-invasive to children. We demonstrated that the levels of 5mC were higher in boys and older children than in girls and younger children, and these findings are consistent with the previous literature[22, 24]. The exact mechanism of sex-specific global DNA methylation is not clear, but it may involve epigenetic regulation of steroid hormone[25].

Iqbal *et al*[26] investigated the global DNA methylation in children aged two to three years old using blood DNA samples and the ELISA method, and found that stunted children had slightly higher levels of 5mC than normal children, albeit not significant. In the present study, where most children were under two years old, the 5mC levels in buccal DNA were significantly lower in severely stunted children than in normal children. The discrepancy may be complicated by increasing global methylation during early childhood[27].

Although the present study is not appropriate for predicting stunting based on 5mC levels, it has been demonstrated that the rapid increase in genome methylation during early normal childhood[27]may be impaired in children with growth problems[28]. Therefore, the levels of global methylation may vary with age[27]. Older children with stunting may also harbor more methylated DNA, because they are more susceptible to bacterial infections, including the development of a poor oral health such as caries[29]. Bacterial DNA also contains 5mC and complicates the analysis using tools such as ELISA that cannot distinguish bacterial from human DNA. It has been estimated that 0.8% of all cytosines in *E. coli* DNA are methylated, compared with about 4% of all cytosine nucleotides in the human genome[30].

In the present study, we showed that the 5mC levels in buccal swab DNA were also higher in boys, which is consistent with other studies using peripheral blood DNA[26] and buccal swab DNA[22]. However, this pattern was not replicated in the mouthwash DNA. The discrepancy between buccal swab and mouthwash DNA may be complicated by the presence of bacterial DNA methylation, which cannot be distinguished by the ELISA method[31].

Stunting is of public health concern because of the risk of low cognitive function with long-term consequences in adulthood[32, 33]. However, in the present study, the difference of cognitive function between normal and stunted children were not statistically significant. Notably, children

living in the village of Kadumaneuh had the most significantly lower cognition scores. However, multivariable regression analysis did not confirm that this difference was statistically significant. Furthermore, mean scores (> 90) of PIQ, VIQ, and FIQ in stunted children were still considered normal in the general population. These findings do not support previous studies showing that stunted children have lower IQ[34, 35]. Koshy *et al*[36] also found that stunted children had significantly lower VIQ than non-stunted children at two, five, and nine years of age. However, these studies did not use the same measures. While Aurora *et al* used Colored Progressive Matrices (CPM) and Koshy *et al* used The Malin's Intelligence Scale of Indian Children (MISIC), an adaptation of the Wechsler Intelligence Scale for Children (WISC), both assessed nine-year-old children[35, 36]. Venables and Raine (2016) examined the intelligence of three-year-old children using the WPPSI subtest and found direct relationships with VIQ and PIQ, while Nasir *et al* used the CPM to measure general cognitive function in four-six-year-old children, and found that height-for-age contributed to cognitive performance only after controlling for sociodemographic background and parental nutrition knowledge[34, 37].

A meta-analysis showed that cognitive ability was highly dependent on social education and a stimulating environment[38]. Although nutritional supplements are important, the risk of low cognitive function may be minimized by providing children with social support. However, a prospective study would be ideal to follow up cognitive performance in school at a later age in this cohort and the association with global DNA methylation or the specific genes affected by methylation using NextGen sequencing.

One study demonstrates that there may be an inverse relationship between the natural variation of 5mC levels and cognitive scores when using buccal swab DNA to determine the contribution of 5mC levels to cognitive performance in children aged four years old[22]. In contrast to this study, we did not find the same inverse relationship for all cognitive parameters. The cause of this discrepancy is currently being investigated, including the small size of our cohort, the use of certain cognitive measurement tools, and the exposure of our cohort to a stimulating environment.

The limitation of our study was that we did not assess dietary intake in our cohort. In addition, we did not analyze breastfeeding history, which may influence DNA methylation[39]. Stunting is primarily the result of chronic malnutrition during the first 1000 days post gestation period. Therefore, low levels of DNA methylation (or global hypomethylation) in a stunted

cohort under the age of two years may reflect low intakes of nutrients containing sufficient methyl donors or cofactors[24]. Since nutritional intervention is critical in the first 1000 days of child development, obtaining a methylation profile using a non-invasive method may identify children younger than two years of age, who require precise nutritional intervention.

Regarding cognitive assessment in stunted children, there have been challenges in assessing preschool children whose attention span is limited. Although the test administrators were trained to observe, build relationships, and carefully administer the task, there were some obstacles to performing the assessment. The high dropout rate of the participants reflected the challenges. Either the children refused to continue the test the following day, or the caregivers took the children to their occasions (*e.g.*, family events), so the children were absent for the second day of assessment. Despite the challenges, assessment of cognitive function is important for early identification of cognitive delays so that appropriate intervention may be implemented earlier.

In conclusion, we demonstrated that both buccal and mouthwash samples were sufficient materials for a follow-up study to clarify the precise molecular mechanisms of an epigenetic alteration affecting cognitive function in stunted children. Stunted children in this cohort may be followed up for further cognitive studies when they reach primary school age.

Fundings

Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia through the Kedaireka 2022 Matching Fund Programme No.282/E1/KS.060.02/2022.

Acknowledgments

The authors would like to thank the participation of mothers and the support of our local community healthcare cadres, research assistants, and YARSI student volunteers in Indonesia. Any opinions, findings, conclusions, or recommendations expressed here are those of the authors alone.

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Figure legends

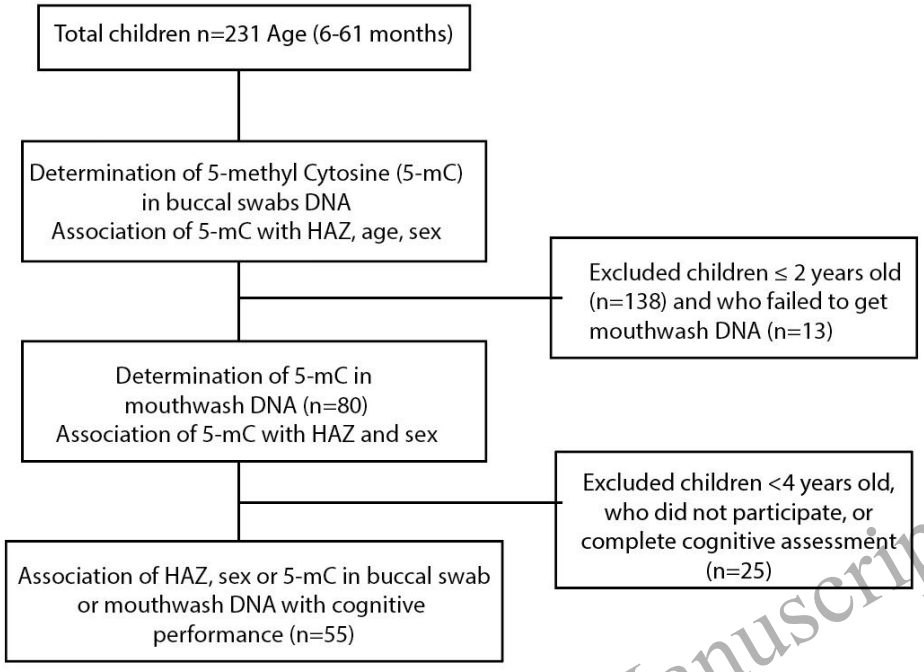

***Fig. 1* Diagram showing subject recruitment, inclusion criteria, and planned analyses.** Abbreviation: HAZ, height-for-age z score.

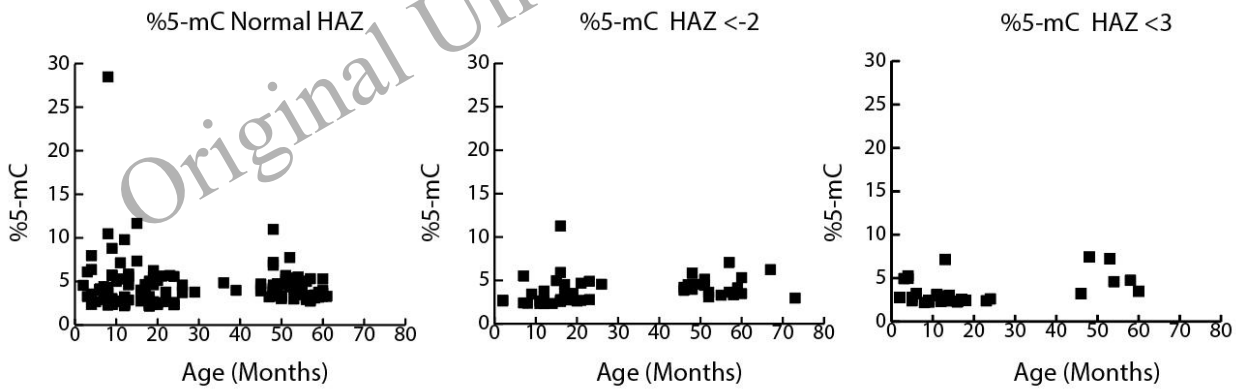

***Fig. 2* Distribution of 5mC levels in buccal swab DNA in different age groups and HAZ status.** Scatter plots to visualize the relationships of 5mC levels on the y-axis and age (months) on the x-axis. Each dot represents a single subject. The left panel represents children with normal HAZ, the middle panel stunting (HAZ <−2), and the right panel severe stunting (HAZ < −3). Abbreviations: HAZ, height-for-age z score; %5mC, percentage of 5-methyl-cytosine.

Tables

Table 1 **Demographics of 231 children aged zero to five years who participated in the present study**

Variables	*n* (%)
Age (months)	
0–24	138 (60)
24–48	14 (6)
≥ 48	79 (34)
Sex	
Boys	131 (57)
Girls	100 (43)
Outcomes	
Normal (−2≤HAZ≤−1)	152 (66)
Stunting (−3≤ HAZ<−2)	49 (21)
Severe stunting (HAZ<−3)	30 (13)
Villages [normal, stunting, severe stunting; *n* (%)]	
Kadubelang (*N* = 73)	47 (31), 17(34), 9 (30)
Kadumaneuh (*N* = 72)	43 (28), 14 (28), 15 (50)
Medong (*N* = 86)	61 (40), 19 (38), 6 (20)

Abbreviation: HAZ, height-for-age z score.

Table 2 **Proportion of the stunting outcome according to independent variables**

Variables	Normal (−2 ≤HAZ ≤ −1)	Stunting (−3 ≤ HAZ < −2)	Severe stunting (HAZ < −3)	*P*-value
Age [months; *n* (%)]				
0–24	87 (57)	27 (55)	24 (80)	0.204 [a]
24–48	9 (6)	4 (8)	1 (33)	
≥ 48	56 (37)	18 (37)	5 (17)	
Sex [*n* (%)]				
Boys	90 (59)	29 (59)	12 (40)	0.140 [a]
Girls	62 (41)	20 (41)	18 (60)	
Villages [*n* (%)]				
Medong	61 (40)	19 (39)	6 (20)	0.776 [a]
Kadumaneh	44 (29)	14 (29)	15 (50)	
Kadubelang	47 (31)	16 (32)	9 (30)	

[a]Chi-square analysis was employed.
Abbreviation: HAZ, height-for-age z score.

Table 3 **Association of 5-methyl-cytosine levels in buccal swab DNA and stunting, age, and sex group**

Variables	5mC level in % [median (IQR)]	*P*
Outcomes		**0.0314**[a]
Normal (HAZ ≥-2)	3.75 (2.80–4.74)	
Stunting (−3 ≤ HAZ <-2)	3.64 (2.70–4.57)	
Severe stunting (HAZ <-3)	2.84 (2.39–4.62)	
Post hoc analysis		
Normal *vs.* stunting		1[b]
Normal *vs.* severe stunting		**0.028**[b]
Stunting *vs.* severe stunting		0.132[b]
Age (months)		
0–24	2.81 (2.53–4.62)	**<0.001**[a]
24–48	4.09 (3.76–4.56)	
>48	4.01 (3.39–4.87)	
Post hoc analysis		
0–24 months *vs.* > 48 months		**< 0.001**[b]
0–24 months *vs.* 24–48 months		**0.038**[b]
24–48 months *vs.* > 48 months		1[b]
Sex		
Boys	3.83 (2.79–4.83)	**0.0187**[c]
Girls	3.25 (2.60–4.48)	

[a]Kruskal-Wallis test was employed to compare the median of multiple groups.
[b]Dunn-Bonferroni was employed as a post hoc analysis.
[c]Mann-Whitney test was employed to compare the median of two groups.
P-values adjusted with Bonferroni correction. Bold font indicates *P*-values < 0.05.
Abbreviations: HAZ, height-for-age z score; IQR interquartile range.

Table 4 **Correlation of age and 5mC levels in different HAZ status**

Outcomes	*n*	*r*	95% CI	*P*
Normal (HAZ ≥ −2)	152	0.1345	−0.02998 to 0.2919	0.0985
Stunting (−3 ≤ HAZ < −2)	49	0.4439	0.1776 to 0.6496	**0.0014**
Severe stunting (HAZ < −3)	30	0.1363	−0.2460 to 0.4819	0.1363

Spearman correlation analysis was performed. Bold font indicates *P*-values < 0.05.
Abbreviation: HAZ, height-for-age z score.

Table 5 **Association of 5mC levels in mouthwash DNA with HAZ and gender in children older than two years old**

Variables	*n*	5mC levels [median (IQR)]	*P*
HAZ			
≥ −2	51	3.68 (3.25–4.27)	
−3–−2	18	3.65 (3.39–4.09)	0.63[a]
< −3	11	3.96 (3.34–4.20)	
Sex			
Boys	47	3.69 (3.26–4.03)	
Girls	33	3.68 (3.38–4.23)	0.59[b]

[a]Kruskal-Wallis was employed to compare median values of 5mC levels in three different HAZ status.
[b]Mann-Whitney was employed to compare median values of 5mC levels in boys and girls.
Abbreviation: HAZ, height-for-age z score; IQR, interquartile range.

Table 6 **Cognitive function according to sex, HAZ, and villages**

Variables	N	PIQ [median (IQR)]	*P*	VIQ [median (IQR)]		FIQ [median (IQR)]	
Sex							
Boys	32	99.5 (86–111)	0.983[a]	100 (95–116)	0.936[a]	101 (94–110)	0.996[a]
Girls	23	100 (92–108)		104 (89–116)		101 (90–113)	
HAZ							
≥ −2	38	100 (85–108)	0.797[a]	101 (94–117)	0.573[a]	101 (94–111)	0.825[a]
< −2	17	99 (92–113)		97 (89–115)		99 (91–112)	
Villages							
Medong	22	102 (97–111)		109 (99–121)		111 (97–115)	
Kadumaneuh	18	97 (80–105)	0.293[b]	93 (85–100)	0.0065[b]	96 (79–103)	0.019[b]
Kadubelang	15	100 (92–108)		105 (98–113)		102 (99–106)	

[a]*P*-value by Mann-Whitney test.
[b]*P*-value by Kruskal-Wallis ANOVA.
Abbreviations: HAZ, height-for-age z score; PIQ, Performance intelligence quotient; VIQ, Werbal IQ; FIQ, Full-Scale IQ; IQR, interquartile range.

Table 7 **Association of 5mC levels in buccal swab and mouthwash DNA, and other variables with cognition**

Variables	PIQ			VIQ			FIQ		
	Association (95% CI)	*P*	R^2	Association (95% CI)	*P*	R^2	Association(95% CI)	*P*	R^2
5mC levels									
Buccal swab DNA[a]	1.33 (−2.02–4.68)	0.42	0.24	−0.21 (−3.63–3.21)	0.90	0.24	0.67 (−2.59–3.93)	0.68	0.24
Mouthwash DNA[b]	9.34 (−14.45–33.13)	0.43	0.07	7.18 (−17.08–31.46)	0.55	0.075	9.47 (−13.66–32.61)	0.41	0.07
Sex (girls)	−0.0625 (−9.14–9.01)	0.98	0.10	3.05 (−6.20–12.31)	0.50	0.10	2.18 (−6.64–11.01)	0.62	0.10
Villages (Kadumaneh)	−6.96 (−18.16–4.23)	0.21	0.36	−11.39 (−22.81–0.03)	0.050	0.36	−9.81 (−20.71–1.08)	0.07	0.36
Stunting outcomes (stunted)	−6.88 (−16.92–3.15)	0.17	0.16	−6.21 (−16.46–4.02)	0.23	0.16	−7.11 (−16.88–2.64)	0.14	0.16

[a]Values of 5mC levels in buccal swab DNA were left untransformed.
[b]Values of 5mC levels in mouthwash DNA were natural logarithm transformed to obtain a normal distribution.
Abbreviations: PIQ, Performance intelligence quotient; VIQ, Verbal IQ; FIQ, Full-Scale IQ.