

ORIGINAL ARTICLE

The Differentiation of Salivary Flow Rate between Stunting and Healthy Toddlers

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ABSTRACT

Introduction: Stunting children experience disruptions in cellular and organ growth and development, which may potentially lead to atrophy of the salivary glands. This research is aimed to find out and analyze the differences between stimulated salivary flow rates in stunting and healthy toddlers. **Materials and Methods:** Quantitative research using the comparative analysis method and the cross-sectional approach. Using a random sampling technique, 30 healthy toddlers and 30 stunting toddlers (27 stunting and 3 severe stunting) aged 3-5 years were selected from Dago Village, Coblong District, West Java. The assessment of salivary flow rate used Saxon Test. **Results:** The salivary flow rate was found lowest in severe stunting toddlers, followed by stunting toddlers, and healthy toddlers showed the highest rate. There are significant differences in salivary flow rates in healthy, stunting, and severe stunting toddlers (p-value=0,015). **Conclusion:** The salivary flow rate in stunting toddlers is lower than healthy toddlers.

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INTRODUCTION

Stunting has been diagnosed as the most common cause of malnutrition. Malnutrition in children occurs due to an imbalance between the supply of nutrients and energy required by the body for growth, maintenance, and specific functions.[1] The nutrition consumed by an individual influences the risk of getting a disease, daily physical activities, and cognitive function. It also has a fundamental role in an individual's growth and development.[2] The malnutrition that frequently occurs in children can lead to impairment of physical growth, specifically reduced height growth rate (stunting).[3]

Based on WHO 2022 data, Indonesia holds the fifth-highest rank in the prevalence of stunting compared to several other countries in Asia.[4] The SSGI (Indonesian Nutrition Status Study) 2022 data revealed that the prevalence of stunting in Indonesia was 21.6%. One out of three toddlers in Indonesia have been found to suffer from stunting, and more than a third (36.1%) of

primary school-age children (7-18 years) in Indonesia were classified as stunting due to chronic malnutrition. [5, 6] Malnutrition or nutritional deficiency as a cause of stunting can have significant impacts on a child's life in both the short and long-term conditions. In the short-term, stunting can adversely affect brain development and intelligence. In the long-term, stunting is not only susceptible to diseases but also have below-normal intelligence levels and low productivity.[7]

According to WHO, a child is categorized as stunting if their height-of-age is below -2 SD and categorized as severe stunting if their height-of-age is below -3 SD.[8] Stunting children are different from stunted children (short stature). The occurrence of short-statured children causes due to some factors, such as hormonal, genetics, malnutrition, and others, while the primary factor of stunting children is lack of nutritional intake during the first 1000 days of life, so that children experience a height less than the average normal child of their age.[9-11] Inadequate nutritional intake might lead to imperfect cell and organ development, such as growth, development, and function of salivary gland.[2] Pregnant women with inadequate nutritional intake during the fourth week of pregnancy are at risk of experiencing disruptions in the growth and development of the salivary glands.[12]

Malnourished children have higher rates of caries and delayed tooth eruption than normal children, low stimulated and unstimulated salivary flow rates were also found.[13] Saliva is a bodily fluid that the function as a self-cleansing agent within oral cavity by clearing remnants of food and bacteria. Individuals with low salivary flow rate are at risk of developing dental caries due to the diminished antibacterial properties of the produced saliva.[13, 14] Another study by Doaa S. Hasheem et al. revealed that the stimulated salivary flow rate in stunting children was lower than normal children, whereas the unstimulated salivary flow rate in stunting children was also lower than normal children, although not statistically significant.[15] A high caries index due to a low salivary flow rate in stunting toddlers can effect ability to eat which can impair adequate nutritional intake. Inadequate nutritional intake can cause disruptions in growth and development, including stomatognathic system. Disruption of the stomatognathic system stunting children can affect to Oral Health-related Quality of Life (OHRQoL) including physical quality, emotional, and social function.[16–18]

Undernutrition such as stunting and micronutrient deficiencies have contributed to the loss of developmental potential among more than 200 million children under five years of age in developing countries, including Indonesia.[19] Developmental disruptions during that age, contribute to lifelong consequences for children, leading to low academic performance, limited economic capabilities, and lifelong disparities. [20] Stunting that occurred in the preschool children can persist into the school-aged.[21]

Previous studies have revealed the decrease of salivary flow rate in stunting children, and it impacted the oral health in children, particularly the occurrence of dental caries that also impact on malnutrition. In the past ten years, studies regarding the analysis of salivary flow differences are still limited and there is no consensus yet on research regarding the differences in salivary flow rate among stunting children. Therefore, this study aimed to discover and analyze the stimulated salivary flow differences between stunting and healthy toddlers.

MATERIALS AND METHODS

Ethical Approval

This study has acquired ethical approval from the Research Ethical Committee of Universitas Padjadjaran (118/UN6.KEP/EC/2023).

Study design

The study type was quantitative research using the comparative analysis method and the cross-sectional approach, which compares the salivary flow rate between stunting and healthy toddlers. Random sampling was used to sampling technique in this study.

Samples

The target population in this study was toddlers aged 3–5, and the reachable population was toddlers aged 3–5 living in Dago, Coblong, Bandung City, West Java. Dago is a locus stunting in Bandung City, which has been established as the focus area for the depletion and prevention of stunting in 2023, with a prevalence of 9.77% (125 children) based on the Bandung Mayor Decision number 050/Kep.2092-Bappelitbang/2022 concerning the Expansion of Locus Area for the Acceleration of Stunting Depletion and Prevention in Bandung City in 2023. This study was conducted in March 2023 at 10 Integrated Healthcare Centers under the Dago Public Health Center; the locations were selected since there are many stunting toddlers aged 3–5.

The nutritional status of the toddlers was double-checked before and after the study using the e-PPGBM and e-Penting applications, with assistance by the Nutritionist of Dago Community Health Center. The obtained data included age, gender, height, and weight recorded over the last three months (January to March). The e-PPGBM application is a community-based online system for nutrition recording and reporting, while e-Penting is an application used by the community and the government in Bandung City for recording stunting-related data. The inclusion criteria were toddlers with stunting, i.e., toddlers whose height-to-age was less than -2 (stunting) and -3 (severe stunting) of the WHO child growth standards median, and healthy toddlers (did not suffer malnutrition whose weight-to-height, height-to-age and weight-to-age equivalent to the WHO child growth standards, in good general health 3 days before data collection, and not currently taking any medications that affect salivary flow) with parental consent to participate. The exclusion criteria were uncooperative toddlers.

The category of stunting toddlers is divided into subcategories based on the decrease in height, namely stunting and severe stunting, and subcategories based on weight loss, namely stunting with underweight and stunting without underweight. Underweight is defined by WHO as children whose weight-for-age is below -2 standard deviations.[22]

Salivary Flow Rate Assessment

The salivary flow rate data retrieval was conducted at 09.00–13.00 Western Indonesian Time (WIB). The subjects were instructed not to eat or drink (mineral water was exceptional) an hour before the retrieval. The subjects were instructed to gargle water before the examination to eliminate food debris. The subjects sit in a relaxed position. The salivary flow rate was assessed using the Saxon Test[23, 24] using instruments and materials accustomed to toddlers subjects. The instruments and materials utilized in this study were 10×10 cm sterile dry gauze (folded at an angle of 90°

into four parts, then folded at an angle of 45° and the ends were folded, resulting in locked folds, and the final size was approximately 24242 cm), cup container (upper diameter of 5.6 cm, height of 3.1 cm, and volume of 50 mL), analytical balance with the accuracy of 10–4, stopwatch, surgical mask, hand gloves, glass for gargling, and clean water.

The subjects were asked to swallow their saliva in their mouth first. Stimulated saliva was accumulated by instructing the subjects to chew weighed dry gauze for 2 minutes. The saliva was spat out at intervals during the chewing to avoid overflowing in the oral cavity. After 2 minutes, the saliva and gauze were spat into a container. The saliva volume was measured by weighing the gauze and the container containing saliva using the analytical balance with an accuracy of 10–4. The result of the weighing was reduced by the initial weight. The weighing was performed at the Integrated Research Laboratory of the Faculty of Dentistry Universitas Padjadjaran. The saliva volume was written in mg and then converted into mL with the assumption of the density of 1.0 g/mL ($V=m/\rho$) (1 gram saliva = 1 mL). Then dividing the saliva volume by the collecting duration to calculate the salivary flow rate.

Statistical Analysis

The analysis determined the correlation between the independent variable (stunting, severe stunting, and healthy toddlers) and the dependent variable (salivary flow rate). A normality test was performed using the Saphiro-Wilk Test; the test result was $p < 0.05$, indicating that the data was abnormally distributed. Therefore, the statistical test used was the Kruskal-Wallis test, a nonparametric test. The data is claimed to be significant if $p < 0.05$. This study also analyzed the correlation between the independent variable (stunting group with and without underweight and healthy toddlers) and the dependent variable (salivary flow rate) using the normality test, i.e., the Saphiro-Wilk; the test result was $p < 0.05$. Therefore, the Kruskal-Wallis was performed as the statistical test.

RESULT

A total of 72 toddlers were involved in this study. However, data from 12 toddlers were deemed invalid due to fluctuating height measurements each month or not meeting the inclusion and exclusion criteria. The total number of subjects analyzed in this study was 60 (30 healthy group and 30 stunting group suitable to the inclusion and exclusion criteria). The stunting toddlers were divided into stunting (45%) and severe stunting (5%) categories. In the stunting group, 11 toddlers was underweight (18%) and 19 toddlers was not underweight (32%).

Table I presents the subjects' characteristics. The subjects' characteristics (age and sex) are displayed in Table I. The number of female subjects (55%) was higher than that of males (45%). Based on age, the number of subjects aged 48–60 months (62%) was higher than those aged 36–47 months (38%).

Table I. The Study Subjects' Characteristics

Characteristics			n	(%)
Healthy group			30	50
Stunting group				
Stunting			27	45
Severe stunting			3	5
Healthy group			30	50
Stunting group				
Stunting without underweight			19	32
Stunting with underweight			11	18
Healthy group	Sex	Boys	14	47
		Girls	16	53
	Age (month)	36-47	9	30
		48-60	21	70
Stunting group	Sex	Boys	13	43
		Girls	17	57
	Age (month)	36-47	14	47
		48-60	16	53

The results of the differences between salivary flow rates of the healthy, stunting, and severe stunting toddlers are analyzed and presented in Table II. The severe stunting toddlers had the lowest salivary flow rate (0.213 [0.054–0.294] mL/minute), followed by the stunting toddlers (0.323 [0.118–0.994] mL/minute), and healthy toddlers showed the highest rate (0.442 [0.196–0.970] mL/minute). The statistical test results showed a significant difference in the salivary flow rate of the healthy, stunting, and severe stunting toddlers, with a score of $p = 0.015$ ($p < 0.05$). The description of the salivary flow rate decline of the healthy, stunting, and severe stunting toddlers is depicted in Figure 1.

Table II. The Analysis Results of the Salivary Flow Rate in this Research

Status	n	Stimulated salivary flow rate (mL/minute)		Kruskal-Wallis Test result
		Range	Median	
Healthy	30	0.196–0.970	0.442	$p = 0.015$
Stunting	27	0.118–0.994	0.323	
Severe stunting	3	0.054–0.294	0.213	

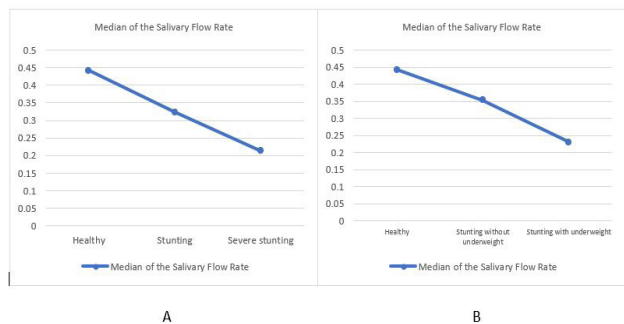


Figure 1: The salivary flow rate in toddlers (A) healthy, stunting, and severe stunting (B) healthy, stunting without underweight, and stunting with underweight.

The results of the differences between the salivary flow rate of the healthy and stunting toddlers with and without underweight are analyzed and presented in Table III. The salivary flow rate of the stunting toddlers with underweight was the lowest (0.230 [0.054–0.510] mL/minute) compared to the stunting toddlers without underweight (0.354 [0.170–0.994] mL/minute) and the healthy toddlers (0.442 [0.196–0.970] mL/minute). The statistical test results showed a significant difference in salivary flow rate in healthy toddlers, stunting toddlers with underweight, and stunting toddlers without underweight, with a score of $p=0.002$ ($p<0.05$). The description of the salivary flow rate decline of healthy toddlers, stunting toddlers with underweight, and stunting toddlers without underweight is depicted in Figure 1.

Table III. The Analysis Results of the Salivary Flow Rate Differences between Healthy, Stunting Toddlers with Underweight, and Stunting Toddlers without Underweight

Status	n	Stimulated salivary flow rate (mL/minute)		Kruskal-Wallis Test result
		Range	Median	
Healthy	30	0.196–0.970	0.442	$p=0.001$
Stunting without underweight	19	0.170–0.994	0.354	
Stunting with underweight	11	0.054–0.510	0.230	

DISCUSSION

The lowest salivary flow rate was found in severe stunting toddlers, followed by stunting toddlers, while healthy toddlers exhibited the highest rate. These findings are parallel with a study by Neetika Singh et al., which was performed on children aged 5–12 and resulted in a significant difference between the stimulated and unstimulated salivary flow rate of malnutrition (determined by the weight-to-age and height-to-age) and healthy children. Children with low height-to-age yielded lower unstimulated salivary flow rate mean (0.14 mL/minute) than healthy children (0.53 mL/minute). The

stimulated salivary flow rate of children with low height-to-age was also lower (1.17 mL/minute) compared to the healthy children (1.94 mL/minute).[13]

Karlla A. Vieira et al. conducted a study on children aged 1–5 (12–71 months old) using the suction method; the study results reported that children with primary malnutrition were classified as very low based on the unstimulated salivary flow rate (0.35 ± 0.14 mL/minute). [25] A study on children aged 3–12 conducted by Hasheem et al. reported that stunting children had a lower stimulated salivary flow rate, with a mean rate of 0.884 ± 0.554 mL/minute, compared to healthy children, with a mean rate of 1.094 ± 0.655 mL/minute. However, the unstimulated salivary flow rate in the study by Hasheem et al. differed from the previous studies; the study reported that the difference was insignificant due to weight alteration of the parotid gland, density reduction of the β -adrenoreceptor, and the parotid gland secreted 50% of the stimulated salivary flow rate and 20% of the unstimulated salivary flow rate.[15]

The saliva production measurement method utilized in studies is various. This study utilized the Saxon Test combined with the spitting and absorbent method to accumulate the stimulated saliva. Saxon Test initiated by Kohler and Winter has been used in previous studies to identify the indication of hyposalivation. This saliva production measurement method is performed in a short period (2 minutes), uses affordable and easily purchased instruments and materials, is more comfortable to the patients, and the use of analytical balance yields a more accurate result compared to the use of measuring glass due to the presence of foamy saliva.

The Saxon Test result is categorized as hyposalivation if the rate is <1.00 mL/minute and non-hyposalivation if the rate is >1.00 mL/minute.[23] This study exhibited that the stimulated salivary flow rates in healthy and stunting toddlers were below 0.5 mL/minute. The salivary flow rates were categorized as hyposalivation. We have not yet found another study conducted on preschool-aged children using the Saxon Test method; hence, we could not compare this study results with other studies. Most previous studies regarding the salivary flow rate of healthy and stunting toddlers were conducted on children above five years old, used various methods, and yielded the stimulated salivary flow rate results of more than 8 mL/minute (categorized as normal rate).

The varied and low salivary flow rates of the healthy and stunting toddlers in this study were caused by the complicity of the Saxon Test performed on the preschool children. Some toddlers swallowed the accumulated saliva and encountered difficulties spitting it. This method can be combined with other methods to aid saliva accumulation in preschool children, one of which is the use of suction. Some toddlers also seemed nervous and anxious while retrieving saliva, and some

were scared of doctors/dentists due to traumatic events while getting injections; hence, the salivary flow rate retrieval process was not performed optimally.

The findings in this study are identical to a study by Neetika Singh et al. which reported that the children salivary flow rate varied in some studies because preschool children had difficulties performing the Sialometry test. There have not been any researchers who provided the normal parameter for toddlers's salivary flow rate.[13] A study by Leonar et al. was conducted to analyze the stimulated salivary flow rate pattern in children (normal height and weight) by six-year longitudinal study; the study resulted in a conclusion that the older a child got (7–12 years old), the higher the stimulated salivary flow rate. The salivary flow rate means when the children aged 7 and 12 were 0.8 mL/minute and 1.8 mL/minute, respectively. At the age of 7, the highest salivary flow rate was 1.1 mL/minute, and the lowest was 0.5 mL/minute.[26]

In this study, 11 out of 30 stunting toddlers suffered underweight. Other studies also reported a correlation between stunting and underweight occurrence. Stunting toddlers might also suffer underweight.[22, 27–29] The three toddlers classified as severe stunting in this study were all underweight. If a child's nutritional intake is severely inadequate, the child may experience severe stunting accompanied by underweight condition. This research showed that stunting-underweight toddlers had the lowest salivary flow median compared to other groups. This finding is parallel to studies by Doaa S. Hasheem et al., Neetika Singh et al., and Siti S et al., which reported that underweight toddlers had lower salivary flow rates compared to healthy toddlers.[13, 15, 30]

The primary factor causing a child to experience stunting is inadequate nutrition needs fulfillment. A study by Weny Wulandary and Trini Sudiarti reported a significant result between inadequate carbohydrate, vitamin A, calcium, and iron intake and stunting. Based on the study result, iron deficiency was the most dominant variable in the occurrence of stunting.[31] A study by Ashraf Soliman et al. discovered that protein intake substantially influenced stunting, which was related to the role of the mTORC1 pathway against the stunting pathogenesis.[2] The nutrition related to the mTORC1 pathway is protein and iron.[2, 32] The mTORC1 pathway acts to control the cell and organ growth that conscious to the presence of amino acids inside the body for protein synthetization. mTORC1 regulates the bone, skeletal muscle, and nervous system development, size determination of an organ, energy balance, and others.[33] A study by Manabu Sakai et al. was conducted *ex vivo* using the rapamycin (mTOR inhibitor)-administered submandibular gland culture and *in vivo* using pregnant ICR rats intraperitoneally injected with rapamycin whose embryos were taken to

be analyzed; the study results discovered that mTORC1 acted in the controlling of submandibular gland morphogenesis. If the mTORC signal was disrupted, it hampered the salivary gland development.[34]

Singh et al. stated that the salivary gland was the organ experiencing atrophy in stunting children with nutrient deficiency (protein, vitamin A, and iron).[13] A study by Ashraf Soliman et al. explained that a low amount of protein in the body caused a decline of essential amino acids circulating in the body compared to normal children; therefore, it impacted the development affected by the mTORC1 pathway, which was known to be sensitive towards the amount of amino acid in the body. The reduction of amino acid in the body leads to mTORC1 suppressing the protein and lipid synthetization as well as cell and organ development.[2] A study by Boyd et al. was conducted on male Wistar mice. The mice were given diets consisting of 85% carbohydrates and without protein, and 2, 9, 27, and 81% casein as the carbohydrate substitute for 28 days. Thirty mice were given water and without food, and ten mice of each group were given the same diet pattern after 28 days. The mice that were not given food drank less water compared to the control group with food, and the mice that had undergone a reduction in food intake of protein were discovered to occur a decrease in salivary gland weight. The decrease of protein synthetization needs impacts the decrease of RNA; hence, the salivary gland shrinks.[35, 36]

Iron deficiency also affects the mTORC1 pathway. Lacking iron consumption might lead to an increase in PP2A activity, which supports the increase of REDD1 gene expression. REDD1 is a small protein whose expression is influenced by environmental stress. The increased REDD1 promotes the dephosphorylation or the Akt inactivation, resulting in the absence of TSC1/TSC2 stimulation. The absence stimulates the Rheb conversion into the form of inactive GDP, resulting in the reduction or the inactivation of mTORC1 and hampering protein synthetization.[32]

The secreted saliva relies on reflex activity and regulated by the autonomic or involuntarily nervous system. The saliva secretion stimulated by the parasympathetic nerve prompts the continuous secretion of saliva in a sufficient amount to maintain the humidity of the mucous membrane and aid the movement of the tongue and lips while speaking. Meanwhile, the saliva secretion stimulated by the sympathetic nerve, which occurs due to stress, causes dry mouth.[37]

Hypofunction of the salivary gland in stunting children have a higher risk of dental caries.[38] Salivary flow rate plays a crucial role in maintaining the oral cavity's defense capacity and cleanliness.[13] The decreased salivary flow rate in stunting children, associated with inadequate protein intake, can impact the production of

Immunoglobulins (sIgA) and cellular immunity. Reduced levels of sIgA are linked to an increased incidence of dental caries. sIgA functions as the primary defense that protects the oral cavity from bacteria causing dental caries, such as *S. mutans*. *S. mutans* bacteria can rapidly convert carbohydrates into organic acids, leading to a low pH environment.[39, 40] However, some studies reveal no significant differences in saliva pH in stunting children.[15, 25] Therefore, further research on saliva pH in this context is warranted.

Suggestions for future studies regard the histopathology test to an atrophy salivary gland of experimental animals which are given a low-nutrition diet, the nutrition pattern of the stunting and severe stunting toddlers, and the salivary flow rate in stunting toddlers with underweight using more proportional samples. The calibration of height measurement by cadre at Integrated Healthcare Center should be re-evaluated to prevent bias in the stunting toddlers's assessment and identification.

CONCLUSION

The stimulated salivary flow rate in the stunting group is lower than the healthy group. Among the toddlers, those classified as severe stunting have the lowest salivary flow rate, followed by the stunting toddlers, and the highest flow rate is observed in the normal group. This research also found that 11 out of 30 toddlers in the stunting group experienced underweight. The salivary flow rate in the stunting group with underweight has the lowest average compared to the stunting group without underweight and the normal group.

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