Original Article

Comparison of Salivary Properties Between Different Degrees of Caries Level in Children Aged Below 72 Months: A Cross-sectional Study

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ABSTRACT

Aim: Early childhood caries (ECC) remain prevalent in many developing countries, including Indonesia. Salivary factors, such as flow rate, pH, and nitric oxide (NO) concentration, are potential indicators of caries risk. The study aimed to compare unstimulated whole saliva (UWS) flow rate, salivary pH, and nitrite ion concentration among caries-free (CF), ECC, or severe ECC (S-ECC) children. **Materials and Methods:** Thirty-one children aged 36–71 months visiting the Pedodontic Department at the Specialized Teeth and Mouth Hospital, University of Indonesia, were categorized into CF, ECC, and S-ECC groups. The UWS flow rate was measured by volume per minute, salivary pH with a universal pH indicator strip, and NO concentration using the Griess reaction. **Results:** The median UWS flow rate was highest in the CF group (0.38 mL/min) and lower in the ECC (0.29 mL/min) and S-ECC (0.33 mL/min) groups. Median salivary pH was 7.00 in CF, ECC, and S-ECC, while NO concentrations were 70.14 µM (CF), 71.04 µM (ECC), and 90.59 µM (S-ECC). None of the differences were statistically significant ($P > 0.05$). The correlation coefficients were UWS flow rate and def-t scores, $r = -0.267$, salivary pH and def-t, $r = -0.066$; and NO concentration and def-t, $r = 0.169$. **Conclusions:** Although trends in the salivary flow rate, pH, and NO concentration were observed, no significant differences were found among caries groups. The small sample size, non-standardized collection, and imprecise pH measurements may have limited the findings. These preliminary studies may support future research on salivary markers in caries risk assessment.

Keywords: *Caries-free, early childhood caries, nitric oxide concentration, salivary pH, severe early childhood caries, unstimulated whole saliva flow rate*

Introduction

Dental caries is a multifactorial infectious disease characterized by the dissolution of tooth minerals, which may lead to pain, infection, nutritional deficiencies, and impairments in learning and speech.[1-3] Despite significant progress in promoting, preventing, and treating oral disease, dental caries continues to impact numerous children.[1,2,4] A 2017 Global Burden of Disease report stated that untreated primary dental caries affected 532 million children worldwide.[5]

Saliva is a critical factor associated with the development and progression of dental caries. It is secreted by the parotid, submandibular, sublingual, and minor salivary glands and is composed of water, electrolytes, enzymes, mucus, antimicrobial agents, and various organic molecules. It maintains oral moisture, aids digestion, and facilitates speech and swallowing.[6]

Saliva helps prevent demineralization and promotes remineralization of the teeth.[7,8] When salivary pH is between 6.8 and 7.2, it becomes saturated with calcium phosphates, which helps rapidly remineralize early

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enamel lesions. However, in more acidic environments, saliva becomes unsaturated, increasing the formation of soluble calcium hydrogen phosphates and the susceptibility of teeth to caries.[7]

Whole saliva consists of salivary gland secretions along with gingival crevicular fluid, epithelial cells, bacteria, food debris, and other oral components. While saliva from individual glands may have specific compositions and properties, whole saliva reflects the integrated oral environments and is thus more relevant to studying complex diseases like caries.[6]

Whole saliva constantly washes the mucous membranes of the larynx, throat, and mouth facilitating swallowing, digestion, and articulation.[4] It also protects the teeth surface and mucous membranes against mechanical, chemical, and biological factors[9] by removing eliminating products of bacterial metabolism, bacteria, and food debris from the oral cavity and the surface of the teeth.[4]

Among its many components, nitrate and nitrite have recently gained attention for their protective roles. Nitrate, absorbed from the duodenum and upper ileum, is reduced to nitrite in the oral cavity. In acidic conditions around dental tissues, nitrite is converted into nitrous acid and nitrogen oxides. Nitrous acid further decomposes into nitric oxide (NO), a reactive radical that contributes to non-immune defense mechanisms in the oral cavity to prevent bacteria from overgrowing.[10]

This study aimed to compare unstimulated whole saliva (UWS) flow rate, salivary pH, and nitrite ion concentration among children who are caries-free (CF), have early childhood caries (ECC), or have severe ECC (S-ECC). We hypothesized that UWS flow rate and pH would be lower and NO concentration higher in ECC and S-ECC children than in CF children.

Materials and Methods

This cross-sectional study used saliva samples from children visiting the Pedodontic Department of the Specialized Dental and Mouth Hospital, University of Indonesia. Sample processing was carried out at the Laboratory of the Oral Biology Department, Faculty of Dentistry, University of Indonesia. A consecutive sampling method was used.

Subjects

Children aged 36 to 71 months were included in the study following written informed consent from their parents or legal guardians. Saliva samples were collected after receiving approval from the Institutional Ethics Committee (60/FKGUI/IX/2024). Based on dental examination findings, participants were categorized into one of three groups: CF, ECC, or S-ECC.

A *post hoc* power analysis was conducted using G*Power (version 3.1; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) to estimate the power achieved for detecting differences in salivary biomarkers among the three groups (CF, ECC, and S-ECC). Based on the median UWS flow rates—0.38 mL/min (CF), 0.29 mL/min (ECC), and 0.33 mL/min (S-ECC)—an estimated effect size (f) of approximately 0.33 was calculated. With a total sample size of 31 ($n = 10$, 10, and 11) and an alpha level of 0.05, the statistical power ($1 - \beta$) was approximately 49%, indicating that larger sample sizes may be needed for future studies.

Dental examination

Intraoral examination was performed using a sickle probe and mouth mirror. Children were included in one of the three groups based on their def-t scores: Group I: CF children (def-t score = 0), Group II: children that have ECC (def-t score of 1–5) and Group III: Children that have S-ECC (def-t score > 5).

Saliva sample collection

UWS was collected from each participant. Children who were able to spit were instructed to do so into a labeled 10-mL collection tube. For those unable to spit, saliva was collected using sterile pipettes and transferred to the same type of tube. Samples were collected between 9:00 AM and 1:00 PM, aligning with patient availability and departmental operating hours. This time window was also selected to minimize the influence of circadian variation on salivary composition as salivary flow rate and pH are generally lowest during early morning hours and gradually increase toward midday.[6] Restricting sample collection to late morning and early afternoon helped reduce diurnal variation between participants, although minor circadian effects within this 4-h window cannot be completely excluded. Children were instructed to abstain from food and drink for at least 1 hour prior to sampling.

Saliva collection was conducted with the child seated upright in the dental chair, head tilted slightly downward, and mouth open. Collection continued until at least 1.5 mL of UWS was obtained, with the time recorded for flow rate calculation. All samples (1.5–2 mL) were immediately placed into a cooler box containing dry ice. The samples were stored at a −20°C laboratory refrigerator up to analysis.

UWS flow rate

The UWS flow rate (mL/min) was calculated by dividing the volume of collected saliva by the time taken to collect it.

Salivary pH

Salivary pH was assessed using a universal pH indicator strip (Dr. Gray Universal Test Paper).[11] The strip

was immersed in the saliva sample, and the pH was determined based on the color change.

Nitric oxide concentration

The NO concentration was measured using the Classical Griess Reaction using a commercially available kit (*Griess Reagent System, Promega Corporation, Madison, WI, USA, Cat # 72536*) incubated for 10 min at room temperature, and the absorbance was measured at 540 nm using an ELISA reader.

Statistical analysis

Data were collected and organized in a Microsoft Excel spreadsheet and then analyzed using IBM SPSS Statistics Version 25 (IBM Corp., Armonk, NY, USA). The normality of the data was assessed using the Shapiro–Wilk test. Data were found to be nonparametric, and measurements are presented as medians and interquartile ranges (IQRs). A Kruskal–Wallis test was used to assess statistical differences in UWS flow rate, salivary pH, and NO concentration between the groups. Spearman's rank correlation coefficient test was used to assess the significance of the relationship between the UWS flow rate, salivary pH, and NO concentration in each group. Statistical significance was defined as $P < 0.05$.

Results

There were 31 children included in the study. The UWS flow rate, salivary pH, and NO concentration was compared between the groups.

Unstimulated whole saliva flow rate

The UWS flow rate in the CF group was 0.32–0.75 mL/min, in the ECC group was 0.12–0.56 mL/min, and in the S-ECC group was 0.16–0.43 mL/min [Figure 1]. Median (IQR) values were 0.38 (0.13), 0.29 (0.18), and 0.33 (0.18) mL/min, respectively. Table 1 The Kruskal–Wallis test indicated no significant differences among groups ($H = 3.887$, $P = 0.143$), with a small effect size ($\eta^2 = 0.067$). Pairwise Mann–Whitney comparisons yielded r values between 0.077 and 0.415, and bootstrap 95% confidence intervals (CIs) for median differences all included zero.

Salivary pH

The salivary pH ranged from 6 to 7 in group CF and group S-ECC, and 7 in group ECC [Figure 2]. Median (IQR) values were 7.00 (1.00), 7.00 (0), and 7.00 (1.00), respectively [Table 1] No significant difference was observed ($H = 4.284$, $P = 0.117$), with a small-to-moderate effect size ($\eta^2 = 0.082$). Pairwise Mann–Whitney comparisons yielded r values from 0.056 to 0.440, with 95% CIs for median differences again overlapping zero.

Nitric oxide concentration

The NO concentration ranged from 42.623 to 218.945 µM in the CF group, 25.575 to 242.298 µM in the ECC group, and 39.704 to 208.757 µM in the S-ECC group. Figure 3 Median (IQR) values were 70.14 (76.82), 71.04 (57.83), and 90.59 (86.68) µM [Table 1]. The Kruskal–Wallis test showed no significant difference ($H = 1.358$, $P = 0.622$) and a negligible effect size ($\eta^2 = -0.023$). Pairwise Mann–Whitney comparisons yielded r values between 0.042 and 0.208 and 95% CIs for median differences indicated no meaningful between-group differences [Table 2].

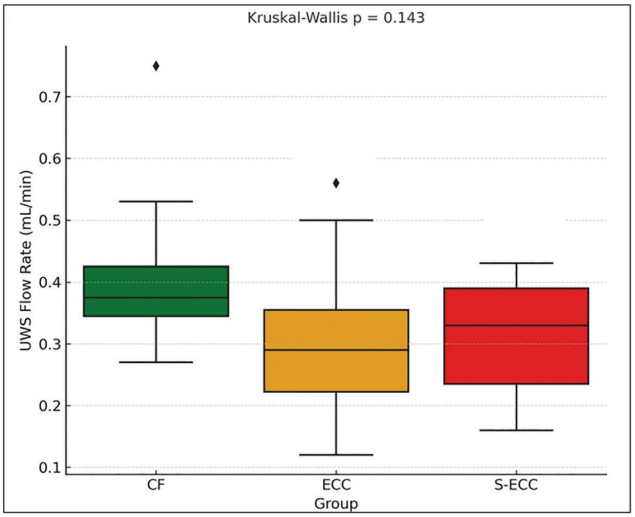

Figure 1: Box plot showing UWS flow rate (mL/min) by caries group (CF = 10, ECC = 10, S-ECC = 11). The median UWS flow rate in the CF group was higher compared to the ECC and S-ECC groups The Kruskal–Walli's test indicated no significant difference among groups ($P = 0.143$; 95% confidence interval)

Table 1: Comparison of median (IQR) UWS flow rate (mL/min), salivary pH, and concentration of nitric oxide (µM) among CF, early childhood caries, and S-early childhood caries

Variables	Sample Group (N = number of samples)			Sig. P Value
	CF $N = 10$	ECC $N = 10$	S-ECC $N = 11$	
UWS	0.38 (0.13)	0.29 (0.18)	0.33 (0.18)	0.143
Salivary pH	7.00 (1.00)	7.00 (0)	7.00 (1.00)	0.117
NO concentration	70.14 (76.82)	71.04 (57.83)	90.59 (86.68)	0.622

UWS = unstimulated Whole Saliva (mL/min), NO = nitric Oxide (µM), CF = caries-free, ECC = early childhood caries, S-ECC = severe ECC

CI 95%, statistically significant at $P < 0.05$ (* means significant variation exists)

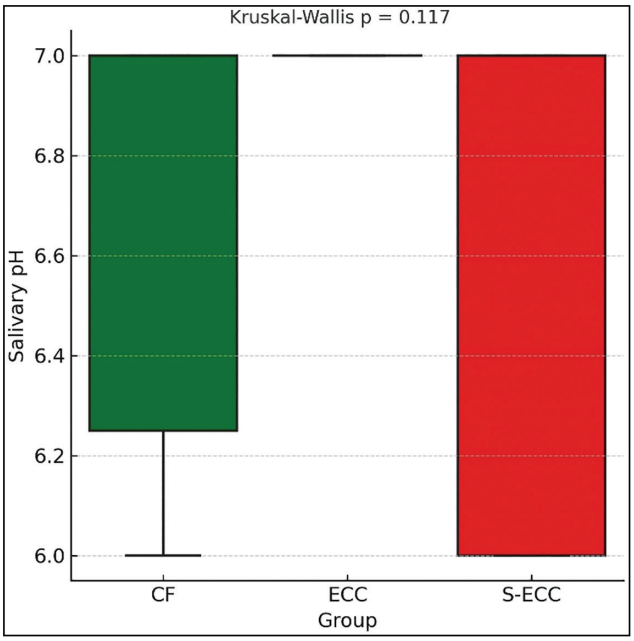

Figure 2: Box plot showing salivary pH by caries group (CF = 10, ECC = 10, S-ECC = 11). The median salivary pH was similar across all groups The Kruskal–Walli's test indicated no significant difference among groups ($P = 0.117$; 95% confidence interval)

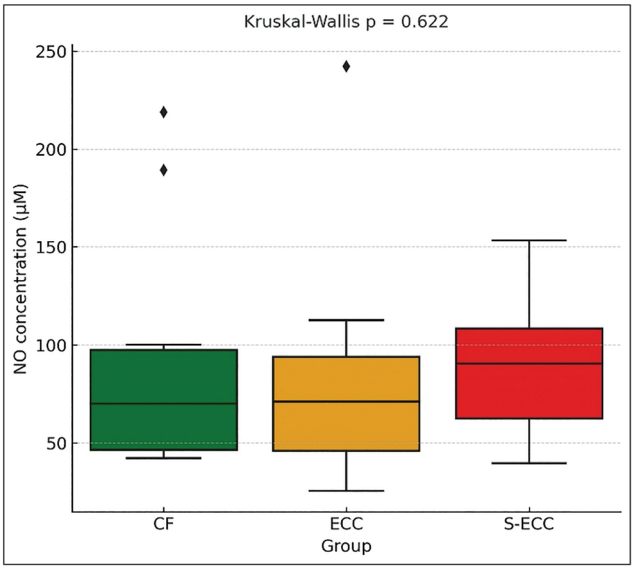

Figure 3: Box plot showing NO concentration (µM) by caries group (CF = 10, ECC = 10, S-ECC = 11). The median NO concentration in the S-ECC group was higher compared to the CF and ECC groups. The Kruskal–Walli's test indicated no significant difference among groups ($P = 0.622$; 95% confidence interval)

Discussion

Saliva plays a vital role in dental caries, a multifactorial disease characterized by the breakdown of hard tissues by oral microorganisms and their metabolites. Functioning as a shield against dental caries, saliva maintains a balance between cariogenic and noncariogenic microbial populations and the interaction of pathologic and protective factors.[1]

Table 2: Correlation between UWS flow rate (ml/min), salivary pH, concentration of nitric oxide (µM), and def-t scores

Variables	Correlation coefficient	Sig. *P* value
UWS	−0.267	0.146
pH	−0.066	0.724
NO	0.169	0.364

UWS = unstimulated whole saliva (mL/min), NO = nitric oxide (µM), CF = caries-free, ECC = early childhood caries, S-ECC = severe ECC
CI 95%, statistically significant at $P < 0.05$ (* means significant variation exists)

This study aimed to compare UWS flow rate, salivary pH, and NO concentration among children categorized as CF, ECC, and S-ECC). We expected to observe a lower UWS flow rate and pH and higher NO concentration in children with ECC or S-ECC than CF children. Although we did not find statistically significant differences, our results provide valuable preliminary insights and highlight areas for further investigation.

The median UWS flow rate was slightly higher in children with CF than in those with ECC and S-ECC, although the differences were not statistically significant ($P = 0.143$). We found a negative, non-significant correlation between salivary flow rate and def-t score, which should be interpreted with caution. These results are similar to findings by Prabhakar *et al.*[12] in Indian children between 7 and 14 years, where CF children were found to have a slightly higher salivary flow rate, although the difference was not statistically significant. Other studies involving children aged between 24 and 71 months[1] and children between 4 and 12 years,[13] reported significantly lower mean salivary flow rates in children with ECC compared with CF children. These differences may be attributed to methodological differences, particularly in saliva collection techniques, participant age, and cooperation levels during sampling.

Salivary parameters, including g flow rate, pH, and certain biochemical constituents such as NO, exhibit circadian fluctuations, with lower values typically observed in the early morning and gradual increases toward midday. We collected samples between 9:00 AM and 1:00 PM to reduce variability associated with extreme circadian phases. Nevertheless, minor intra-window fluctuations in salivary composition cannot be completely excluded and should be considered when interpreting the results. Future studies may benefit from standardizing collection at a narrower time point to further control for diurnal variation.

In our study, the method of saliva collection (spitting vs. pipette) was adapted to each child's ability, which introduced variability in flow rate measurement. Some younger children struggled to spit effectively or tended to swallow saliva during collection, requiring assistance with a pipette. We acknowledge that this variability may have introduced random errors and affected the accuracy

of the flow rate estimation. We recommend the use of a standardized collection method in future research.

The median salivary pH was similar in the CF (7.00 [1.00]), ECC (7.00 [1.00]), and S-ECC (7.00 [0]) groups and without statistical significance ($P = 0.117$). A weak negative correlation was observed between salivary pH and def-t score ($r = -0.066$), which was not statistically significant ($P = 0.724$). A study done by Abe *et al.* in Nigeria[14] and Sivakumar and Narayanan in India[1] found lower pH values in the ECC groups. Sivakumar and Narayanan[1] found a statistically significant correlation between pH and the number of decays. Deshpande *et al.*[15] also found the CF Group had a significantly higher mean salivary pH value than that of the S-ECC Group in children from the age group 3–6 years. In contrast, our pH results did not show such a pattern, possibly due to the use of a universal pH indicator strip, which provides only coarse pH estimates in full-unit increments, in contrast to more precise instruments like saliva-specific strips or digital pH meters. This measurement limitation likely contributed to the lack of significant findings and has been explicitly acknowledged.

We found the highest NO concentration in the S-ECC group, although this difference was not statistically significant. Some studies reported similar trends,[16,17] while others observed higher NO levels in CF children,[18,19] suggesting a potential preventive role. These discrepancies may reflect methodological differences or variations in age, hygiene, and diet. Despite the lack of significance, the observed trend supports further investigation into NO as a potential biomarker.

The relatively small sample size ($N = 31$) in our study may have limited the ability to detect statistically significant differences. Although the sample size was consistent with previous exploratory research, it may have been insufficient given the biological variability of salivary parameters. Additionally, the lack of strict control over collection timing and pre-collection conditions (e.g., circadian rhythm and fasting status) may have introduced confounding variables. Despite these constraints, the findings provide useful exploratory data for evaluating salivary biomarkers in relation to caries severity.

Given the cross-sectional nature of this study, it is not possible to infer causality from the observed associations. Future longitudinal studies are recommended to monitor changes in UWS flow rate, pH, and NO concentration over time and to better elucidate potential causal pathways. Additionally, this study did not control for participants' dietary patterns or oral hygiene practices, both of which are known to influence salivary NO levels and pH. These uncontrolled factors may have introduced variability in the biomarker measurements and should be addressed in future research.

Despite the lack of statistically significant findings, the clinical relevance of this study cannot be overlooked. The trends observed, such as reduced salivary flow rate in children with ECC and S-ECC, slightly lower pH in the S-ECC group, and higher NO concentrations in more severe caries cases, suggest subtle shifts in the oral environment that may contribute to caries risk. In particular, reduced unstimulated salivary flow may reflect early functional changes that impair natural cleansing and buffering capacity. Similarly, variations in NO concentration may reflect underlying microbial changes or inflammatory responses within the oral biofilm.

These findings reinforce the idea that salivary biomarkers, when interpreted as part of a broader risk assessment model, may serve as a useful parameter for the early identification of children at a higher risk of ECC. Although none of the individual parameters proved to be reliable standalone predictors, their combined trends may still inform preventive strategies in pediatric dentistry. Such information is particularly important in young children, in whom early intervention can significantly influence long-term oral health outcomes. Thus, while this study is exploratory, it supports further research incorporating larger, more diverse cohorts and refined methodologies to validate the use of noninvasive salivary diagnostics in clinical practice.

Conclusions

This study explored the differences in UWS flow rate, salivary pH, and NO concentration among CF, ECC, and S-ECC children. Although trends were observed—namely, lower flow rates and pH in children with caries and higher NO concentrations in those with more advanced disease—none of the differences reached statistical significance.

These findings indicate that UWS flow rate, salivary pH, and NO concentration, when evaluated individually, may not be sufficient to reliably distinguish caries risk in young children. The lack of significant differences may be partially attributed to methodological limitations, including the small sample size, variability in collection methods, broad timing of sample collection, and use of imprecise tools for pH measurement.

Future research involving larger and more diverse populations, along with standardized collection protocols and more precise analytical methods, is essential to better elucidate the role of salivary biomarkers in caries development. In clinical settings, these salivary parameters may still hold value as part of a broader risk assessment model that includes behavioral, dietary, and microbial factors.

Acknowledgment

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Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Patient Declaration of Consent

Before the data collection process started, a consent form was sent to the parents, asking for their consent for their child to participate fully in the study.

Ethical policy and Institutional Review board statement

The study is approved from Institutional Ethics Committee (60/FKGUI/IX/2024)..

Patient declaration of consent

Not applicable.

Authors contributions

PNG: Conceptualization, methodology, data collection, data analysis, data interpretation, discussion of the results, conclusion, writing-original draft, reviewing and editing the manuscript. EWB: Conceptualization, methodology, data analysis, data interpretation, discussion of the results, conclusion, writing-original draft, reviewing and editing the manuscript, and provided supervision. BMB: Conceptualization, data analysis, data interpretation, discussion of the results, conclusion, and provided supervision. MFR: Conceptualization, discussion of the results, conclusion, and provided supervision. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Data availability statement

Data is available on resaonable request by communicating corresponding author mail.

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