

Biological Factors in 2 – 3 years old Children in Determining Risk Factors of Early Childhood Caries: Pilot Study

Asty Samiaty Setiawan¹, Risqa Rina Darwita^{2*}, Sri Susilawati³,
Diah Ayu Maharani⁴, Ariadna Adisattya Djais⁴

1. Doctoral Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia Jakarta 10430, Indonesia.
2. Department of Preventive and Public Health Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia.
3. Community Dental Health, Faculty of Dentistry, Padjadjaran University, Bandung 40132, Indonesia.
4. Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia.

Abstract

Damage on the primary teeth can be used as the best predictor of permanent tooth damage. ECC is a serious societal health problem in developing and industrial countries and can begin in the early stage of life and develop rapidly in children with high caries risk. The aim of this study was to analyze biological factors in 2-3 years old children in determining risk factors for early childhood caries.

We conducted a cross-sectional study with samples of children, aged 2–3 years, who lived in selected villages with active Integrated Service Post in the Bandung City Community Health Center working area. Caries examination was performed as baseline data using the WHO standard check form to get information dmfs and oral hygiene was assessed using the Oral Hygiene Index Simplified (OHI-S). Microbiological examination was done to calculate the number of colonies of bacteria *Streptococcus mutans* on colony forming units (CFU). We performed statistical testing using the Chi-square test, Mann-Whitney Rank Sum Test and Spearman's rho.

The total sample of the study was 156 children with caries prevalence of the was 69.2 % (n = 108) of the children had ECC and the mean (\pm SD) dmfs was 8.1 (\pm 12.1). The majority of children with ECC showed oral hygiene in the fair category with the mean (\pm SD) debris index of 1.12 (\pm 0.76). The average number of *Streptococcus mutans* in boys ($3.5E+04 \pm 3.8E+04$) was more than that of girls ($2.5E+04 \pm 2.9E+04$), where dmfs in boys were higher than girls.

Oral hygiene and *Streptococcus mutans* colony have a significant correlation to the occurrence of early childhood caries, the direction of positive correlation with the strength of correlation was included in weak category.

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Introduction

Dental caries causes pain, impairs eating and sleeping and causes local and systemic infections. Damage on the primary teeth can be used as the best predictor of permanent tooth damage.^{1,2} The American Academy of Pediatric Dentistry (AAPD) describes Early Childhood Caries (ECC) as a condition where ≥ 1 of the

primary teeth is damaged (perforated or non-perforated lesion), missed (due to caries), or filled on the tooth surface in children aged <71 months or 6 years old.³⁻⁶ ECC is a serious societal health problem in developing and industrial countries and can begin in the early stage of life and develop rapidly in children with high caries risk.⁶⁻⁸

Oral diseases in Indonesia, of which caries is the main problem, tend to increase over time. The Indonesia Basic Health Research 2013 report showed an increase in caries prevalence from 43.4% in 2007 to 53.2% in 2013. The rates of oral diseases in children aged 1–4 years were 10.4% and were 18.9% in children aged 5–9 years.⁹ City of Bandung and District of Indramayu, caries prevalence was noted at 96% while

*Corresponding author:

Risqa Rina Darwita
Department of Preventive and Public Health Dentistry
Faculty of Dentistry, Universitas Indonesia
Jakarta 10430, Indonesia
Email: risqarina2004@yahoo.com

prevalence of odontogenic infection was slightly higher in the District of Indramyu (78.7%) than in the City of Bandung (75.1%).¹⁰ Research conducted in Mekargalih Village, Jatinangor Subdistrict, Sumedang Regency, Indonesia, showed that approximately 70.24% of children aged 3–5 years suffered from odontogenic infections due to untreated dental caries.¹¹

ECC has a complex etiology and is considered a multifactorial disease. It is also associated with hypoplastic enamel malnutrition and unmaintained oral hygiene in developing countries. *Streptococcus mutans*, maternal education, family structure, and social status also demonstrated a prominent role as causes of ECC.¹² ECC develops early in life. It is important to establish the relationship between the children's social, behavioural and biological factors in early life and ECC so that effective measures can be implemented at appropriate times to prevent ECC.¹³

The above descriptions have encouraged us to perform preliminary studies in the form of clinical examinations of children aged 2–3 years, the aim of this study was to analyze biological factors in 2-3 years old children in determining risk factors for early childhood caries.

Materials and methods

We performed a cross-sectional study from samples of children aged 2–3 years living in selected villages with active Integrated Service Post (Posyandu) in the Bandung City Community Health Center (Puskesmas) working area, according to the predetermined population criteria. The sample selection was performed using non-probability sampling with the purposive sampling technique. The sample size was determined based on the total sampling according to the criteria of the predetermined population. The inclusion criteria were the child in good health, no systemic abnormalities and willing to oral examinations. The exclusion criteria included children have abnormalities in their mouths so they cannot open their mouths and taking antibiotics for a long time. Based on the ethical agreement by the Research Ethics Committee (KEPKG) of the Faculty of Dentistry of the University of Indonesia, with registered number 75/Ethical Approval/FKGUI/XII/2017, informed consent was obtained from the parents before the 2- to 3-year-olds were examined.

Clinical and microbiological examination

Clinical examination of all the children received conducted by a two trained examiner with the aid of WHO CPI probe and disposable mouth mirrors. Caries examination in the 2- to 3-year-old children was performed as baseline data using the World Health Organization (WHO) standard check form to get information decay, missing and filled surface (dmfs) from occlusal, mesial, buccal and distal of the primary teeth.¹⁴ An established oral debris index was used to assess the amount of debris on primary teeth. The amount of debris measured in the OHI-S are on the labial surfaces of four teeth numbers and the lingual surfaces of two teeth numbers. The DI score is obtained by the sum of the debris score for all teeth, divided by the number of surfaces scored. Furthermore, to give clinical relevance to the index, the oral cleanliness is considered good if the DI-S score is between 0.3- 0.6; as fair when it is 0.7 - 1.8; or poor when the score is between 1.9 to 3.0.^{15,16} Cohen's Kappa was used to assess the intra-examiner agreement on the assessment.¹

Microbiological examination was done to calculate the number of colonies of bacteria *Streptococcus mutans*. The first stage with sterilization of equipment and materials used in oral biology laboratory.¹⁷ Plaque samples were collected by swabbing all surfaces of the anterior teeth with sterile cotton tips.¹³ The swabs were cut off was input to a 1.5 ml eppendorf tube containing 1 ml of NaCl 0.9 % solution as much as 1 ml, the solution was homogenized and then taken as much as 0.5 ml and put into a 1.5 ml eppendorf tube containing 0.5 ml NaCl 0.9 % as much as 0.5 ml, then took the solution which has been homogenized by 0.3 ml. The solution 0.3 ml of was put into a petri disk containing a selective medium TYSB20. TYSB20 (Tryptone Yeast Cysteine Bacitracin 20 Sucrose Extract) was a selective medium for select and identify the bacteria *Streptococcus mutans*.¹⁷ Then the selective media was put into an incubator at 37 °C for 1-2 x 24 hours, after that using the colony counter was done calculation of the number of colonies with colony forming units method (CFU / ml).¹³

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS). Bivariate analysis for dependent variables (ECC) and independent

variables (age, gender, oral hygiene, *Streptococcus mutans* count) were prepared. The statistical tests used included the Chi-square test, Mann-Whitney Rank Sum Test and Spearman's rho. The statistical significance level for all tests was set at 0.05.

Results

The total sample of the study was 156 children aged 2 – 3 years old who came with their mothers to the Integrated Service Post in the work area of the Community Health Center, then their mothers gave consent for clinical examination. 50.6% of children aged 2 years and 49.4% of children aged 3 years were examined for dmfs, Debris Index and *Streptococcus mutans* colonies. Kappa value for inter-examiner agreements for the presence of dental caries was 0.87 and debris index was 0.92.

The overall caries prevalence of the study sample was 69.2 % (n = 108) of the children had ECC, while the mean (± SD) dmfs was 8.1 (± 12.1). The children were not caries detected was 30.8 % (n = 48). Chi-square analysis showed there was significant (p < 0.05) between gender and child's age with ECC (Table 1).

	n	No Caries Detected (%)	ECC (%)	x ²	p-value*
Gender					
Boys	85	20 (23.5)	65 (76.5)	4.59	0.032
Girls	71	28 (39.4)	43 (60.6)		
Child's Age					
2 year	79	31 (39.2)	48 (60.8)	5.39	0.020
3 year	77	17 (22.1)	60 (77.9)		
Oral hygiene					
Good	34	19 (55.9)	15 (44.1)	17.11	0.000
Fair	80	24 (30)	56 (70)		
Poor	42	5 (11.9)	37 (69.2)		
Total	156	48 (30.8)	108 (69.2)		

Table 1. Association between gender, child's age, oral hygiene and early childhood caries. *Chi-square test (significant p < 0.05).

Boys had a higher caries prevalence (76.5 %), while the mean dmfs 9.8 (± 14.6) compared than girls (60.6 %) while the mean dmfs 5.9 (± 7.9). Dmfs scores between boys and

girls were not significantly different (p > 0.05). Three year old children had a higher caries prevalence (77.9 %), while the mean dmfs 10.6 (±14.8) compared than two year old children (60.8 %), while the mean dmfs 5.7 (±8.5). Dmfs scores between 2 year old and 3 year old were significantly different (p < 0.05) (Tabel 2).

Oral hygiene was assessed by the accumulated debris and plaque on the primary teeth. The majority of 2 – 3 years old children with ECC showed oral hygiene in the fair category with the mean (± SD) debris index of 1.12 (± 0.76). Chi-square analysis showed there was significant (p < 0.05) between oral hygiene and ECC (Tabel 3). Debris Index between boys 1.24 (± 0.76) and girls 0.98 (± 0.73) were significantly different (p < 0.05). Debris Index between 2 years old 1.04 ± (0.77) and 3 years old 1.21 (± 0.76) were not significantly different (p > 0.05) (Tabel 2).

Mean value and standart deviations for *Streptococcus mutans* counts (CFU) in 2 - 3 years old children was 3.1E+04 ± 3.5E+04. The average number of *Streptococcus mutans* in boys (3.5E+04 ± 3.8E+04) was more than that of girls (2.5E+04 ± 2.9E+04), where dmfs in boys were higher than girls. The average number of *Streptococcus mutans* in children of 2 years old (3.4E+04 ± 3.7E+04) was lower than children of 3 years old (2.7E+04 ± 3.3E+04), where children of 2 years old were lower than children of 3 years old. *Streptococcus mutans* counts between gender and age were not significantly different (p > 0.05) (Tabel 2).

	dmfs		<i>Streptococcus mutans</i> in CFU		Debris Index	
	Mean±SD	p-value**	Mean±SD	p-value**	Mean±SD	p-value**
Gender						
Boys	9.8±14.6	0.56	3.5E+04±3.8E+04	0.212	1.24±0.76	0.035
Girls	5.9±7.9		2.5E+04±2.9E+04		0.98±0.73	
Child's age						
2 year	5.7±8.5	0.012	3.4E+04±3.7E+04	0.289	1.04±0.77	0.193
3 year	10.6±14.8		2.7E+04±3.3E+04		1.21±0.76	
Total	8.1±12.1		3.1E+04±3.5E+04		1.12±0.76	

Table 2. Mean (±SD) dmfs, *Streptococcus mutans* in CFU, Debris Index and components for the total sample by gender and child's age.

The results of the analysis showed that there was a significant correlation between ECC and Debris Index and *Streptococcus mutans* in CFU. The significance value between ECC and index debris was 0.000 with Spearman correlation value of 0.420 which indicates that the direction of positive correlation with weak correlation strength. The significance value between ECC and *Streptococcus mutans* in CFU

was 0.011 with a Spearman correlation value of 0.203 which indicates that the direction of positive correlation with weak correlation strength.

	Debris Index		<i>Streptococcus mutans</i> in CFU	
	r	p-value*	r	p-value*
Early Childhood Caries	0.420	0.000**	0.203	0.011***

Table 3. Correlation between early childhood caries with Debris Index and *Streptococcus mutans* in CFU. *Spearman's rho, **Correlation significant at the 0.01 level, ***Correlation significant at the 0.05 level

Discussion

Dental caries was one of the most challenging diseases faced by children globally and efforts continue to identify prevention strategies to limit the burden of this disease at both individual and population levels.¹⁸ This study showed that the prevalence of ECC children aged 2 - 3 years in Bandung City was more than 50% (69.2 %). Caries may occur shortly after the eruption of primary teeth, in 2–3 years of age. The incidence continues increasing with age and reaches a peak in children 5–6 years of age.¹⁹ The incidence of caries in this study also showed a significant correlation with age. The difference of caries incidence in two and three age groups of children was significant. Similar to the results of the previous studies, present study also showed that caries prevalence increased significantly with age.^{20,21} The severity of caries, as reflected by the values of dmfs, was increased with age.¹² The finding that caries experience and the number of confirmed cases of ECC increased with increasing age was as expected, because there is an increasing number of erupted primary teeth which become exposed to the oral environment and cariogenic challenge. Also, as children grow older there was a change in the dietary habits and hygiene practices.²²

Gender in this study also became an observation of ECC occurrence. This study found that boys of 2 – 3 years old were more likely to be affected by ECC; this observation has been previously reported in a study of children aged 2-5 years in the City of Zagreb, Croatia.²³ However, several studies of Aboriginal and non Aboriginal children investigating gender as a

factor influencing early childhood caries development did not find a significant difference. Similarly, that boys in both age groups were more likely to be affected by dental caries, contrary to a previous study of Canadian Aboriginal children which found no difference between gender and contrary to previous reports which show that females usually have more caries experience than males of the same age. This observed gender difference was also found in a parallel study of surveyed 7- and 13-year-olds from this population. The reason for more boys being affected by ECC and dental caries was not known at this time.²⁴

Chi-square analysis determinant that the presence of oral hygiene children of 2 – 3 years old showed there was significantly ($p < 0.05$) associated with the presence of ECC. A positive trend was evident, however, indicating that 3-year-olds with ECC had a higher mean debris index score. While this association does not identify causation and effect, it does allow one to speculate that those children with increased plaque at the time of examination likely had debris on their teeth at the time caries activity began. It also indicates that children with ECC continue to suffer from less-than-ideal oral hygiene.¹⁵ An alarming finding was the high occurrence of dental plaque among these 1- to 3-year-olds; the present figures exceed those of some recent studies. Although the prevalence of ECC for the present 12 to 19 month-olds was rather low, the high occurrence of dental plaque indicates their increased risk for future caries calling for imperative procedures to reduce that risk factor.²⁵ Another study showed the presence of visible plaque was not only associated with the prevalence of ECC but is also an indicator of the early stages of caries and the severity of disease. Reported that visible plaque on the incisors was highly correlated with ECC in 18-month-old children. These results emphasize the importance of plaque control in very young children.²⁶

There are a number of factors thought to contribute to dental caries, including concentrations of *Streptococcus mutans* bacteria. The average number of *Streptococcus mutans* colonies counted in this research was $3.1E+04 \pm 3.5E+04$. This result was lower than the result of a study conducted by Smiech-Slomkowska et al. in 2007, which stated that the number of *Streptococcus mutans* in 1-year-old children was

2.9E+06. Levels of *S. mutans* exceeding 10^6 of bacteria per 1 ml of saliva indicate a high caries risk.¹⁷ Early colonisation of *Streptococcus mutans* was a major risk factor for ECC as well as future dental caries. Al-Otaibi et al. had performed a longitudinal assessment for *Streptococcus mutans* colonisation and dental caries in 2-4-years-old children, and discovered that children who harboured *Streptococcus mutans* in their plaque at the age of 2 had the most caries activity by the age of 4.¹⁸

However, even though the number of *Streptococcus mutans* in this study was found in the low category, but it still needs important attention, because the research of Al-Otaibi et al. has stated that one observed limitation of the bacteriological evaluation of caries risk was that the relationship strength between *mutans* counts and caries was affected by the medium employed.¹⁸ In addition to microbiological factors, many other salivary elements are also involved in the incidence of caries. Salivary pH and buffering capacity are also play an important role in caries development.¹⁹

There was also found significant correlation between number of *Streptococcus mutans* and the ECC status, with the p-value of 0.011 ($p < 0.05$). *Streptococcus mutans* was viewed as the principal bacterial species initiating dental caries. High levels of *Streptococcus mutans* in plaque and early colonization with *Streptococcus mutans* can contribute to the development of ECC.⁶ Several previous cross-sectional studies that used a regression model found that mutans streptococci was a significant factor for the presence of ECC.²⁷

Spearman correlation value of 0.203 which indicates that the direction of positive correlation with weak correlation strength. The salivary *Streptococcus mutans* count as the predictive values alone were not sufficient as a determiners of caries activity. Past caries experience and educational levels of parents were also needed to be analysed in order to reach validity in caries prognosis.²⁸ The study conducted by Twetman et al. stated that clinical examination of past caries experience may be useful in the assessment of caries risk for children and, therefore, in the planning of preventive measures. Oral health education and the use fluoridated dentifrices have proven to be effectively improved the oral health status of these children.²⁹

The results above indicated that the occurrence of ECC was influenced by several factors, where in this study, knowledge factors and the number of *Streptococcus mutans* were slightly related to ECC, so that other behavioural and risk factors need to be investigated further.

Conclusions

The results of this study showed that 3-year-olds children have a higher ECC prevalence compared to 2-year-olds children. Oral hygiene and *Streptococcus mutans* colony have a significant correlation to the occurrence of early childhood caries, the direction of positive correlation with the strength of correlation was included in weak category.

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Declaration of Interest

The authors report no conflict of interest.

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