

# Comparison of Some Salivary Characteristics in Iraqi Children with Early Childhood Caries (ECC) and Children without Early Childhood Caries

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**Background:** Early Childhood Caries (ECC) is a unique form of caries that develops in temporary dentition. It has a multifactorial infectious disease. Saliva is one of the most important factors, which has an important protective effect against tooth decay when its multiple characteristics and functions are normal. The study aimed to compare some salivary characteristics in children with ECC and children without ECC.

**Materials and Methods:** Case-control, cross-sectional observational study. The 77 pre-schoolers aged 37 to 72 months (12 with ECC, 26 with ECC-S, and 39 without ECC) examined and we collected the stimulated saliva. The pH microelectrode was used to determine pH and buffer capacity; a formula that involves volume, collection time, and specific saliva weight was used to test the salivary flow rate. The potentiometric and phosphate methods were used to determine fluoride through spectrophotometric, colorimetric absorption techniques.

**Results:** The results got to show that there are no statistically significant differences in pH, buffer capacity, salivary flow rate, and levels of fluoride and phosphate, in children with ECC, ECC-S, and without ECC. The risk factors, such as mother's education, bottle use, brushing frequency, and previous dental care of the child are more important at the time of developing ECC than some salivary variables.

**Conclusion:** The risk factors, such as mother's education, bottle use, brushing frequency, and previous dental care of the child are more important at the time of developing ECC than some salivary variables such as pH, buffer capacity, salivary flow rate, and levels of fluoride and phosphate.

**Keywords:** early childhood caries, fluoride, saliva, phosphate, buffer capacity, tooth brushing

## Introduction

Dental caries is the most common chronic diseases in many countries because they are a serious Public Health problem because of their high prevalence, impact on individuals and society, American Association of Dental Paediatrics adopted the term "Early Childhood Caries" (ECC) for specific caries modality of temporary dentition, which affects infants and children in preschool age and develops immediately after the first teeth erupted.<sup>1</sup> Dental caries is a chronic pathology with an infectious component occurs both in enamel sub-surfaces and in deeper dental tissues such as dentin and dental pulp, induced by pH variations that lead to the imbalance between demineralization and remineralization in enamel.<sup>2</sup> Although

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ECC is classified as a chronic and infectious disease caused by *Streptococcus mutans*,<sup>3</sup> the influence of other factors such as saliva can change how dental tissues react to acidic wastes produced by bacteria.<sup>4,5</sup>

The present study aims to describe and compare some salivary characteristics such as pH, buffer capacity, fluoride and phosphate levels, and salivary flow rate in children with ECC versus children without ECC.

## Early Childhood Caries (ECC)

Dental caries can occur in early childhood stages affecting primary dentition being commonly known as Early Childhood Caries<sup>1</sup> (AAPD, 2003). This caries had initially called “bottle caries”, to refer to a severe form of caries associated with the use of the bottle. In the next two decades, it does not relate the improper eating habits to the stereotypical medical appearance, and that caries was an infectious disease. Therefore, the AAPD recommended the term of ECC to better reflect its multifactorial etiology.<sup>6</sup> The AAPD defined the ECC as: “Presence of one or more carious tooth surfaces (with or without cavity lesion), lost (because of caries) or blocked in any primary tooth of a child between birth and 71 months of age”. They specify it that in children under 3 years of age, any sign of smooth-surface caries is an indicator of severe ECC (ECC-S). From 3 to 5 years of age, 1 or more cavitated smooth surfaces, lost (because of caries), or clogged in the anterior maxillary temporary teeth index  $\geq 4$  (at 3 years of age),  $\geq 5$  (at 4 years of age), or  $\geq 6$  (at 5 years of age) corresponds to ECC-S. The concepts of Early Childhood Caries and Early Severe Childhood Caries have been used for over ten years to describe the state of caries present in children under 6 years.<sup>7</sup>

## ECC Clinical Characteristics

The ECC has a characteristic clinical pattern. The development of their lesions is rapid and affects according to the chronology of dental eruption. Cysts begin in the dorsal third of the upper anterior parts of the vestibular head. It then affects the occlusal surface of the first molars, the upper and lower canines, and then the second molars. It does not affect the lower central incisors in most cases, because of the flow of the sublingual glands and the position of the tongue during suction, which acts as a protective mechanism.<sup>8</sup> However, they compromised in the more advanced stages. There are four stages in the development of the ECC.

Early Childhood Caries has severe repercussions at the local and systemic levels. With the progression of the disease and the development of carious lesions without interruption, the most common immediate consequence is pain, which can affect the child's daily activities. There are difficulties in eating and sleep disturbances, which cause a delay in the child's physical development in weight and height. There is also an increase in emergency care and hospitalizations and the cost of treatments. Similarly, ECC is a cause of school absenteeism and decreased cognitive abilities. There is a decrease in their quality of life because of these health problems.<sup>9</sup>

## Saliva

Saliva is a complex and mixed secretion, which has an important protective effect against tooth decay when its characteristics of buffer capacity, cleaning effect, antibacterial action, and maintenance of calcium and phosphate levels are normal. And a pathological effect, which favors the appearance of carious lesions, when these characteristics altered or diminished.<sup>5</sup> Many research studies recognize that saliva can a diagnostic fluid. Not only does it show the susceptibility to caries of an individual, however, the recognition of physiological and pathological changes that are reflected in saliva also implemented.

The relationship between Saliva and Caries: The effects of saliva on the development of dental caries depending on the amount and composition of its secretion. It is of considerable importance to recognize and determine the participation of saliva in the demineralization's modulation and remineralization process of dental structures exposed to the oral cavity.<sup>10</sup>

To describe the protective power that saliva possesses against the carious process, it is convenient to summarize it in the following aspects: First, saliva plays a role in the elimination of microorganisms and food residues present in the mouth. After carbohydrate intake, the concentration of sugars in saliva at rest increases exponentially and this stimulates the secretory response of the salivary glands causing an increase in salivary flow. The sugar progressively diluted thanks to the increase in salivary flow. This leads to the volume of saliva in the mouth returning to its resting levels.

## Relationship Between Saliva and ECC: There are Few Studies in the Literature That Associate ECC with Saliva

Bagherian and Asadikaram<sup>11</sup> compared some salivary characteristics among children with ECC and children

without ECC and concluded that children without ECC had higher pH levels and better buffer capacity.

Preethi et al<sup>12</sup> examined the relationship between certain salivary influences in children with cavities and without caries. We also worked with non-stimulated saliva in this study and we deduced from it that there was a decrease in salivary flow, pH, calcium, and buffer capacity among children with caries.

Pannunzio et al<sup>13</sup> found a relation between salivary parameters and dental caries such as children with obesity and overweight had increases in phosphate, sialic acid, protein levels, and peroxide activity it favored the situations for the development of dental caries.

## Review of Literature

There are few published studies to assess pre-school ECC prevalence. Mella et al,<sup>14</sup> in 1996, studied the prevalence of bottle caries and some risk factors in 1260 children aged 2 to 4 years in three cities, with different concentrations of fluoride in drinking water. Prevalence in Iquique of 2.5%, in Valparaíso of 6.4%, and Santiago of 12.2%, and they got an average of caries in these cities of 7.8%.

Echeverría et al<sup>15</sup> published in 2003 a study that showed the prevalence of ECC in preschoolers of the JUNJI gardens of the Metropolitan Region. A significant sample of 179 children of a medium-low socioeconomic level examined. The result got revealed that 33.72% of the children presented this pathology.

## Hypothesis

There are significant differences in the characteristics of salivary composition in children with ECC versus children without ECC.

## Main Aim

Establish whether there are significant differences in the characteristics of salivary characteristics in children with ECC versus children without ECC.

## Specific Objectives

- (a) Determine salivary variable levels in children with ECC and ECC-S and without ECC.
- (b) Compare salivary variable levels among children with ECC versus children without ECC.
- (c) Establish simple relationships between some salivary variables (pH, buffer capacity, salivary flow rate, and fluoride and phosphate levels) with risk

factors for ECC (mother's education, bottle use, previous dental care child, and brushing frequency).

## Materials and Methods

### Type of Study

The methodological design of the study corresponds to a cross-sectional case-control observational study. The total number of children examined was 77 (12 with ECC, 26 with ECC-S, and 39 without ECC). The sample under study corresponded to the 37 to 72-month-old children who attended the "pediatric preventive dentistry clinic/collage of dentistry\Mustansiriyah University". Finally, a comparison of dependent means used for the sample size calculation, getting a minimum sample of 34 subjects, both for the study and control group (Satterthwaite test).

### Inclusion Criteria

Children of both genders, from 37 to 72 months, Children with tutors who agree to take part in the study, by signing an informed consent cooperating child that allows clinical examination and subsequent saliva collection.

### Exclusion Criteria

Uncooperative children attending to the pediatric preventive dentistry clinic/collage of dentistry\Mustansiriyah University do not allow the examination and/or collection of saliva. Children with systemic diseases and/or pharmacological treatment.

### Information Collection Instruments

For each patient, the required information was recorded in a clinical registry specially planned for this investigation. This information included patient identification data, odontogram, and diagnosis of the child.

The study and clinical examination were performed by a previously calibrated operator for the diagnosis of ECC. An inter-examiner calibration was carried out and performed by three examiners, in two stages. In the first instance, they analyzed a digital document to unify diagnostic criteria, by reviewing the bibliography and diagnosing photographs. Subsequently, 10 children from 36 to 72 months of age were examined by visual examination. The Kappa statistical test was performed to quantify the degree of concordance of the three examiners, where the minimum value required is 0.80. The Kappa value got was 0.92, indicating a high inter-examiner agreement.

These 10 children examined in postgraduate had a saliva collection, which was subsequently stored in sterile tubes at ( $-20^{\circ}\text{C}$ ) for analysis. In the morning at 8–11 o'clock, all samples were taken at the same time. These saliva samples were used to measure the five salivary variables understudy, to calibrate the operator with the use of instruments. In the first visits, we gave informed consent to all the parents of the children between 37 and 72 months of age. Once the consents were signed, we worked with the children. We examined each child individually, writing their data in their respective clinical file registry, and subsequently submitted to the saliva test.

We did salivary stimulation using sugar-free gum for children. They chewed the gum for a few minutes and then sterile test tubes were used to collect the stimulated saliva. Then, these tubes are stored at ( $-20^{\circ}\text{C}$ ) for analysis. We took all samples at the same time in the morning at 8–11 o'clock.

Then, in the laboratory, we measured blindly each salivary variable in the following manner and order:

1. Salivary pH: we used pH meter WTW pH 537 with a pH electrode for determination of pH, that previously calibrated between pH 4 and 7.
2. Buffer capacity: The Ericsson Method is the classic standard method for determining the buffer capacity in saliva. We took 0.5 mL aliquot of centrifuged saliva and 1.5 mL of 0.005 M HCl added thereto, and the mixture stirred for 20 minutes to remove the  $\text{CO}_2$ . Finally, we measured pH with the same pH electrode previously calibrated.

**Salivary Flow Rate:** We numbered and weighed all sterile test tubes using a Radwag WTB 2000 scale. Then after saliva collection, we again weighed these tubes. For the calculation of salivary flow velocity (SFV), the following formula applied expressing its result in mL/min:

$$SFV = \frac{(S2 - S1)/1.005}{T}$$

Where:

S2 = Tube weight with saliva

S1 = Empty tube weight

T = Collection time

1.005 = Specific weight of saliva (g/mL).

Subsequently, 3mL of each salivary sample took to three different Eppendorf tubes, then put in the centrifuge at 13,000 rpm for 90 seconds.

1. Fluoride: We used the selective ion method for fluoride level determination. The ionometer used was the Oakton Ion 510 Series, previously calibrated with standard solutions of 0.1 ppm, 1 ppm, and 10 ppm which would allow measurements in those ranges. We added 0.5 mL of TISAB II to 0.5 mL of centrifuged saliva, stirred them for 5 minutes, and, subsequently, the concentration of salivary fluoride read on the ionometer.
2. Phosphate: Spectrophotometric colorimetric absorption technique was used to measure phosphate. Salivary phosphate forms a phosphomolybdate with sodium molybdate, which by reduction becomes molybdenum blue, determined by spectrophotometry, and which is proportional to the amount of salivary inorganic phosphate present. Before the use of the spectrophotometer, a calibration curve Absorbance v/s phosphate concentration (Figure 1) was constructed, using the UNICAM UV/VIS Spectrometer UV2 spectrophotometer.

We took 0.2 mL of centrifuged saliva, then mixed it in a flask with 1.0 mL of reagent 1 and 0.2 mL of Reagent 2 and 3, allowing it to stand for 15 minutes at room temperature. Subsequently, 2 mL of reagent 4 added, mixed, and finally flushed with 10 mL distilled water. We transferred this solution to a plastic cuvette for spectrophotometry, measuring absorbance at 750nm.

### 1. Operation of Variables

**Early Childhood Caries:** qualitative nominal variable (present/absent). It will be considered as the present to the dental clinical examination of one or more carious surfaces (cavitated and non-cavitated lesions), lost (because of

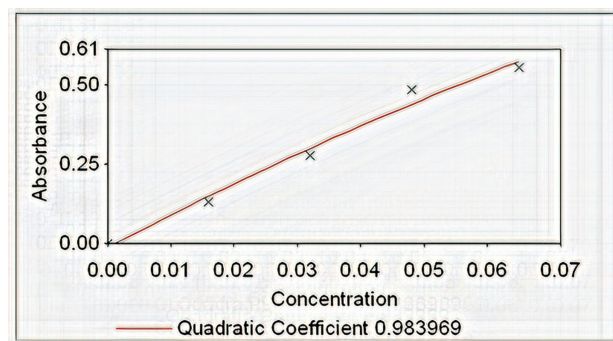


Figure 1 Calibration graph.



caries) or blocked (in primary dentition) in children 24 to 72 months of age, a concept accepted by the AADP<sup>1</sup> (AAPD, 2003).

The educational level of the mother: qualitative ordinal variable (less than 12 years/12 years/over 12 years) declared by the mother.

Bottle use: nominal categorical variable (use/do not use) according to the suggestion of classification used by the WHO.

Brushing frequency: ordinal qualitative variable ( $\geq 2$  times a day/ $< 2$  times a day) declared by the mother.

Prior dental care: nominal categorical variable (yes/no) according to the mother's statements.

PH: continuous quantitative variable determined by the use of a pH microelectrode.

Buffer capacity: quantitative variable determined by the simplified system based on the Ericsson method.

Salivary flow rate: a continuous quantitative variable that was evaluated by weighing the saliva collection tube before and after finishing saliva collection, expressing its values in mL/min through a formula that involves the collection time and weight specific to saliva.

Fluoride: quantitative variable. Fluoride determination was carried out by potentiometric methods using fluoride specific ion electrode.

Phosphate: quantitative variable determined through spectrophotometric, colorimetric absorption techniques.

## Statistical Analysis

The analysis of data was performed using the computer software IBM® SPSS® version 21 (USA). The exploratory data analysis was performed with a calculation of means, standard deviation, minimums, and maximums for quantitative variables. The Shapiro–Wilk test was used to determine the normality of the data distribution. All salivary variables measured did not show normal distribution. Therefore, the Mann–Whitney test was used for comparisons between the groups of children with ECC and without ECC. Kruskal–Wallis test was used for comparisons between groups of children with ECC-S, ECC, and without ECC.

For the determination of statistical significance between ECC and risk factors such as mother's education, bottle use, previous dental care, and brushing frequency, the Chi2 test was used.

To determine the association between risk factors and the prevalence of ECC, the calculation of OR (Odds Ratio)

was performed through multivariate logistic regression analysis.

Statistical significance was considered if the amount of P-value  $< 0.05$ .

## Results

**Characteristics of the Sample:** The total Iraqi children examined in this study were 77 who aged (37 to 72) months.

**Distribution by gender:** Of the total children examined, 34 were female, corresponding to (44%), while the total of a male was 43 corresponding to (56%).

**Distribution by age:** The average total age of the sample was  $52 \pm 9.6$  months. The most frequent group was 49 to 60 months.

**ECC prevalence:** Of the 77 children examined, 38 suffered from ECC (49.35%) and 39 did not present ECC (50.65%).

**Prevalence OF ECC, ECC-S, and children without ECC:** Of the 77 children examined, 12 had ECC (15.58%), 26 ECC-S (34%), and 39 did not have ECC (50.65%).

**Distribution according to mother education:** Of the 77 children examined, 36 of them had mothers with higher education (47%), 33 had mothers with full secondary education (43%), 1 with a mother with incomplete secondary education (1%), and 7 had mothers with Complete Basic Education (9%).

## Assessment of Salivary Features in Children with ECC and Children without ECC

### pH

The pH in children with ECC was slightly lower than in healthy children. Children with ECC presented an average of pH 6.94 ( $\pm 0.82$ ) and those without ECC 6.99 ( $\pm 0.67$ ). When comparing salivary pH between the group of children with ECC and children without ECC, no significant differences were observed ( $P = 0.672$ ) (Table 1).

### Buffer Capacity

Children with ECC had a buffer capacity of 5.16 ( $\pm 1.15$ ), while healthy children had a 5.07 ( $\pm 1.10$ ). Table 1 shows that there were no significant differences ( $p = 0.575$ ) when analyzing the buffer capacity between both groups studied.

**Table 1** Average Values of Salivary Characteristics in Children with and without EC

Group	pH	Buffer Capacity	SFR	F	Phosphate
ECC	6.94 ± 0.82	5.16 ± 1.15	1.29 ± 0.93	0.031 ± 0.013	2.56 ± 0.98
Healthy	6.99 ± 0.67	5.07 ± 1.10	1.28 ± 0.75	0.033 ± 0.014	2.75 ± 1.02

### Speed of Salivary Flow

The salivary flow rate was 1.29 ( $\pm$  0.93) (mL/min) in the group of children with ECC, and in healthy children, it was 1.28 ( $\pm$  0.75). When comparing this salivary variable between both groups there was also no significant difference ( $p$  = 0.285).

### Fluoride

Children with ECC had an average of 0.031 ( $\pm$  0.013) of salivary fluoride (ppm), with no significant difference ( $p$  = 0.290) with the group of children without ECC, whose salivary average of fluoride was 0.033 ( $\pm$  0.014).

### Phosphate

As seen in [Table 1](#), there were no significant differences ( $p$  = 0.355) in phosphate levels (mmol/L) in children with ECC, whose average was 2.56 ( $\pm$  0.98) while in children without ECC was 2.75 ( $\pm$  1.02).

## Assessment of Salivary Features in Children with ECC, ECC-S, and Children without ECC

Because there were no significant differences in the salivary variables measured among children with and without ECC, a comparison between three groups of children with ECC, ECC-S, and without ECC was done.

### pH

The average salivary pH of children with ECC was slightly higher than in healthy children and with ECC-S. Children with ECC had an average pH of 7.03 ( $\pm$  0.75), while those with ECC-S had an average of 6.90 ( $\pm$  0.86), and healthy children 6.98 ( $\pm$  0.67). When comparing salivary pH between the group of children with ECC,

ECC-S, and children without ECC, no significant differences were observed ( $p$  = 0.904).

### Buffer Capacity

Children with ECC-S had a buffer capacity of 5.11 ( $\pm$  1.08), while children with ECC had the highest value of 5.27 ( $\pm$  1.34), and healthy children the lowest of 5.07 ( $\pm$  1.10). [Table 2](#) shows that there were no significant differences ( $p$  = 0.719) when analyzing the buffer capacity between the three groups studied.

### Speed of Salivary Flow

The salivary flow rate was 1.20 ( $\pm$  0.90) (mL/min) in the group of children with ECC-S, the lowest value of the three groups. Children with ITC had the highest VFS value of 1.45 ( $\pm$  1.03) (mL/min), while healthy children had an intermediate value of 1.28 ( $\pm$  0.75). When comparing this salivary variable between the three groups there was also no significant difference ( $P$  = 0.323).

### Fluoride

Children with ECC-S had on average 0.033 ( $\pm$  0.014) of salivary fluoride (ppm) and those with ECC 0.034 ( $\pm$  0.017), with no significant difference ( $p$  = 0.422) with the group of children without ECC, whose salivary average of fluoride was 0.033 ( $\pm$  0.014).

### Phosphate

As seen in [Table 2](#), there were no significant differences ( $p$  = 0.295) in phosphate levels (mmol/L) in children with ECC-S, whose average was 2.83 ( $\pm$  1.04), showing the level highest of the three groups. Healthy children showed an intermediate value of 2.75 ( $\pm$  1.02), and children with ECC the lowest values of 2.23 ( $\pm$  0.73).

**Table 2** Comparison of Salivary pH, Buffer Capacity, SFR, F, and Phosphate Levels in Children without ECC, with ECC and ECC-S

Group	pH	Buffer Capacity	SFR	F	Phosphate
Healthy	6.98 ± 0.67	5.07 ± 1.10	1.28 ± 0.75	0.033 ± 0.014	2.75 ± 1.02
ECC	7.03 ± 0.75	5.27 ± 1.34	1.45 ± 1.03	0.034 ± 0.017	2.23 ± 0.73
ECC-S	6.90 ± 0.86	5.11 ± 1.08	1.20 ± 0.90	0.033 ± 0.014	2.83 ± 1.04

## Bivariate Analysis Between ECC and Risk Factors

As showed in Table 3: A bivariate analysis was performed between risk factors and ITC through OR (Odds Ratio) and Fisher's exact test, with a significance level of  $p < 0.05$ . The results showed a significant association of ECC with the use of a bottle, with the mother's education ( $\leq 12$  years of study), the child's previous dental care, and the frequency of daily brushing.

## Multivariate Analysis with Logistic Regression

A multivariate logistic regression analysis was finally carried out from a complete model to determine the association between ECC and the various risk factors evaluated.

The most significant risk factor in the model was the mother's education, followed by the frequency of brushing and the use of a bottle (Table 4). Children whose mothers have a lower level of education ( $\leq 12$  years of study) are 14 times more at risk of ECC. Using a bottle and a lower frequency of brushing ( $< 2$  times a day) increases the risk of having ECC tenfold. With prior dental care, we observed the opposite direction in the association estimate. As shown in Table 3.

## Discussion

The present study aimed to compare some salivary characteristics in a group of Iraqi children with ECC and without ECC. The results obtained show that there are no statistically significant differences in pH, buffer capacity,

salivary flow rate, and levels of fluoride and phosphate, in children with ECC, ECC-S, and without ECC.

The results gained show that the average pH in the group of children diagnosed with ECC (7.03) was slightly higher concerning children without ECC and ECC-S (6.98 and 6.90 respectively), not being statistically significant. This coincides with the results got by Preethi et al<sup>12</sup> in 2010, show that the average pH in the group of children diagnosed with ECC (7.03) was slightly higher concerning children without ECC and ECC-S (6.98 and 6.90 respectively), not being statistically significant. Where the Achmad et al<sup>25</sup> in 2019 shows most preschool children suffer from ECC with the most risk factor being very acidic PH. High acidity causes many dental caries and Fiyaz et al<sup>16</sup> in 2013. It should be noted that the pH results gained in this study are within normal ranges (6.8–7.5), which would show that both children with and without ECC have a pH above the critical enamel, thus hindering the demineralization process and the production of new carious lesions.

In relation to the buffer capacity, there were also no significant differences in the three groups studied, with an average of 5.07 for healthy children, 5.27 for those with ECC, and 5.11 for children with ECC-S. All values fall within a buffer capacity range considered low (4.00–5.74), which could denote that the three groups are more likely to develop decay. Preethi et al<sup>12</sup> also found no significant differences in buffer capacity and even proposed that factors such as microbial flora, diet, and food retention may dominate buffering capacity. Bagherian and Asadikaram<sup>11</sup> in 2012 manifested the contrary in their

**Table 3** Association of Risk Factors with ECC Through Bivariate Analysis

Risk Factor	OR	Confidence Level (95%)	P-value
Bottle use	11.2	3.4 to 38.6	0.0001
Mother's education ( $\leq 12$ years old)	17.8	5.0 to 66.8	<0.0005
Brushing frequency	13.4	3.6 to 60.3	<0.0005
Previous dental care	3.5	1.1 to 9.9	0.0084

**Table 4** Logistic Regression Analysis Between Risk Factors and ECC

Risk Factor	OR	Confidence Level (95%)	P-value
Bottle use	10.6	2.3 to 49.7	0.003
Mother's education ( $\leq 12$ years old)	14.2	3.0 to 66.3	0.001
Brushing frequency	10.7	2.1 to 55.1	0.004
Previous dental care	0.5	0.5 to 9.5	0.294

study, where caries-free children presented a buffer capacity significantly greater than that of children with ECC. However, considering that there was no major difference between children with and without ECC in this study, buffer capacity, as a single factor, has a low correlation with caries activity, which supports the theory that ECC is a disease of multifactorial etiology.

The salivary flow rate (SFR) was normal ( $> 1$  mL/min) in all the groups studied, being 1.28 mL/min for healthy children, 1.45 mL/min for children with ITC, and 1.20 for children with ECC-S. These results coincide with the studies by Ghanim et al<sup>17</sup> in 2012, where both established that there is no statistically significant difference in the salivary flow rate (SFR) between subjects with and without caries. Zainab and Salih<sup>18</sup> in 2012 explained the opposite, showing that a group of subjects with caries had lower SFR concerning a control group. The literature shows that there is a direct correlation between salivary clearance and SFR. Subjects with reduced SFR show higher levels of *Lactobacilli* and *Streptococcus mutans* than those with normal SFR, which could increase the risk of developing caries lesions. Because the groups in this study had similar values of SFR, it may be suggested for future research to analyze the levels of *Streptococcus mutans* and *Lactobacilli* as salivary variables, to see if there is no difference in the levels of microorganisms between a group of subjects with cavities and without cavities. The fluoride concentration varies according to the amount and concentration of fluoride ingested, either by the systemic or topical route. The average salivary fluoride in the group of children with ECC-S and without ECC was 0.033 ppm, while the group of children with ECC had a slightly higher concentration of 0.034 ppm, not being statistically significant, suggesting that this variable Saliva is also not a determining factor in the development of dental caries. Souza et al<sup>19</sup> in 2014, also did not find a correlation between caries and fluoride levels, however in his study fluoride concentrations were higher, 0.134 ppm in the group of subjects with high carious activity and 0.156 ppm in the group with low caries levels. These higher concentrations may be for multiple factors, such as the fluoridation of water in that region and/or the intake of fluoride-rich foods. Zero<sup>20</sup> in 2006 also measured levels of salivary fluoride and compared them in subjects with higher and lower caries activity, determining that those with lower caries activity had statistically higher levels of fluoride, so we suggest it more studies measuring salivary fluoride.

The present study did not show significant differences in salivary concentrations of inorganic phosphate. Children

with ECC had an average of 2.23 mmol/l in stimulated saliva, while those with ECC-S and without ECC had higher concentrations, 2.83 and 2.75 respectively. Multiple studies agree with these results such as Mohammed and Sultan<sup>21</sup> in 2007, and Bagherian and Asadikaram<sup>11</sup> in 2012, which also determined the low correlation between concentrations of salivary inorganic phosphate and dental caries. However, Fiyaz et al<sup>16</sup> in 2013 stated otherwise, showing that a group of subjects with caries had lower phosphate levels than those without caries. Although the importance of phosphate precipitation on the dental surface is well known in re-mineralization processes, the concentration of these ions would not be an indicator of risk for caries susceptibility, according to the results.

The literature has determined multiple risk factors for ECC. In this study, we evaluated four factors to see its correlation with ECC: mother's education, bottle use, brushing frequency, and the child's previous dental care.

The results acquired show that children, whose mothers do not have higher education ( $\leq 12$  years of study), are 17 times more at risk of suffering ECC. The multivariate analysis, when analyzing the 4 risk factors with the ECC, suggests that this variable is the most important and determining factor for the ECC. These findings are consistent with multiple studies, such as that of Qin et al<sup>22</sup> in 2008, where they also associated the years of studies of the mother with the risk of presenting ECC.

In multiple studies, the bottle use has been named, such as González<sup>23</sup> in 2003, as a risk factor for ECC. In this study of the 38 children diagnosed with ECC and ECC-S, 27 of them still took a bottle.

Oral hygiene in this study had the expected relevance, both in the bivariate and multivariate analysis, finding an association between ECC and oral hygiene habits (daily brushing greater than or equal to 2 times). Children who brush their teeth less than 2 times a day are 13 times more at risk of presenting ECC. In the multivariate analysis, it turned out to be the second most important variable in the development of ECC. There are other studies, such as Harris et al<sup>24</sup> in 2004, whose results showed the opposite. This may be due to an information bias (courtesy bias) typical of any survey. People respond to what they believe is right or what the professional wants to hear, hiding accurate information.

International publications associate the low prevalence of ECC with early visits to the dentist. In this study of the 38 children with ECC and ECC-S, 26 of them had not had prior dental care. The results got to show that those



children who have never attended the dentist have 3 times the risk of presenting ECC, this variable is statistically significant among the group of children without ECC and with ECC. However, in the multivariate analysis, it turned out to be significant in the opposite direction than expected, indicating to be the least important of the four factors analyzed. This suggests that when analyzing the four variables together, it is the least significant for the development of ECC, however prior dental care may be essential to prevent the onset of the disease, as long as it is done at the corresponding times, before that the disease is already established.

The results are significant because there are national studies regarding ECC and salivary variables. It is the first study in Chile that analyses and compares salivary characteristics among children with and without ECC. Regarding the results, we suggest that the salivary variables measured in this study are less relevant for the development of ECC than risk factors such as mother's education, bottle use, previous dental care, and hygiene habits. The higher or lower prevalence of caries does not depend so much on the salivary characteristics of a child but the aforementioned risk factors.

The limitations of this study are that no other salivary variables were measured, such as the presence of *Streptococcus mutans*, *Lactobacilli*, biological salivary factors, etc. that could be associated with ECC. In addition, only stimulated saliva was used instead of non-stimulated; the latter is the one that predominates in the oral cavity during the day, being perhaps more representative of the physiological state of the oral cavity. However, this would imply difficulty when collecting it in preschool children at such a young age. Other limitations are the sample size and the study population. For future studies we suggest it work with a greater number of children, coming from a single educational establishment and from the same socioeconomic stratum, to have a more representative and homogeneous sample.

The present findings highlight the need for strategies aimed especially at prevention, taking into account that the high prevalence of ECC could lead to decay in permanent dentition, with its consecutive effects on the health of individuals. It is essential to educate mothers concerning oral hygiene habits and bottle use and, finally, to emphasize the importance of the child's dental controls at an early age.

## Conclusions

Although there were no statistically significant differences in pH, buffer capacity, salivary flow rate, and levels of

fluoride and phosphate, in children with ECC, ECC-S, and without ECC. The risk factors associated with ECC, such as mother's education, bottle use, brushing frequency, and previous dental care of the child are more important at the time of developing ECC than some salivary variables.

## Ethical Approval

This study "Early Childhood Caries and its consequences in the integral development of the child" has been approved by the Mustansiriyah Scientific Ethical Committee of the Faculty of Dentistry (Protocol 54, February 27, 2019).

The study was carried out following the principles of the Declaration of Helsinki. We invited each participant to be a part of the study, informing them about the clinical measurements and surveys to be applied, and their potential risks and benefits. The agreement was registered through an informed consent endorsed by the Ethics Committee of the Faculty of Dentistry.

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## Disclosure

The authors report no conflicts of interest for this work.

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