







# Association of salivary parameters and erosive tooth wear in preschool children

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**Abstract:** The aims of this study were to 1) assess the association between erosive tooth wear (ETW) according to the BEWE (Basic Erosive Wear Examination) scoring system and salivary parameters and 2) compare salivary parameters according to ETW severity in the subgroup of children with ETW. This cross-sectional study included 52 preschool children aged 5 years paired by sex. A calibrated examiner assessed ETW using BEWE criteria, and stimulated saliva was collected to determine salivary flow, osmolality, pH, and buffering capacity. The children were divided into two groups: without ETW (n=26; BEWE score 0) and with ETW (n=26; BEWE scores 1 and 2). Logistic regression analysis was used to determine the magnitude of the association between ETW and salivary parameters and estimates of odds ratios (OR). In the unadjusted analysis, the ETW group was more likely to have lower salivary flow (OR = 0.079; 95%CI = 0.013–0.469; p = 0.005) and lower osmolality (OR = 0.993; 95%CI = 0.985–1.000; p = 0.049). In the adjusted analysis, salivary flow remained significantly associated with ETW (OR = 0.087; 95%CI = 0.014–0.533; p = 0.008). Lower salivary osmolality values were observed in ETW, especially in preschool children with more severe ETW (BEWE score 2). Receiver operating characteristic (ROC) determined a salivary osmolality cutoff point of < 201 for the presence of ETW. In conclusion, salivary flow rate was significantly associated with ETW prevalence. Lower values of salivary osmolality were observed in preschool children with distinct erosive lesions with significant loss of tooth structure (BEWE 2).

**Keywords:** Saliva; Child; Tooth Erosion; Tooth, Deciduous.

## Introduction

Dental erosion is characterized by a progressive loss of hard dental tissues caused by acids and not involving bacteria.<sup>1</sup> It is a multifactorial condition, involving intrinsic and extrinsic acid sources.<sup>2</sup> The development and progression of erosive tooth wear (ETW) are related to chemical, biological, and behavioral factors.<sup>1,3-5</sup> Some of these factors, such as saliva and acquired pellicle, may influence the progression of ETW.<sup>6</sup>

Saliva plays an important role in ETW progression. The development and progression of ETW depend on salivary pH, flow, and buffer capacity.<sup>1,3-5</sup> Salivary osmolality is a promising measure that reflects the individual oral hydration and saliva viscosity, and has been



correlated to some oral diseases.<sup>7,8</sup> Few studies have assessed the specific relationship between ETW and salivary factors in deciduous teeth.<sup>9,10</sup> In addition, no previous study has investigated the influence of saliva osmolality on ETW.

According to a meta-analysis, ETW in preschool children is considered a pathology as is dental caries.<sup>11</sup> It should be mentioned that the enamel of deciduous teeth is less mineralized, more permeable, and has a lower thickness than permanent teeth, being more susceptible to ETW.<sup>12-14</sup> Regarding deciduous dentin, it is not clear whether this substrate is more prone to erosive tissue loss than permanent dentin.<sup>15</sup>

In this context, the severity of ETW in deciduous teeth should be assessed early to guide the clinical management of the patient. Although some studies have evaluated ETW in deciduous teeth using the BEWE (Basic Erosive Wear Examination) scoring system,<sup>16-18</sup> none assessed the association between ETW prevalence and salivary parameters in preschool children.

Therefore, the aims of this study were 1) to assess the association between ETW according to the BEWE scoring system and salivary parameters and 2) to compare salivary parameters according to ETW severity in the subgroup of children with ETW. The main conceptual hypothesis was that ETW prevalence is associated with salivary parameters in preschool children.

## Methodology

This study was conducted according to the STROBE checklist recommendations.

### Ethical Statement

The study protocol was approved by the local Research Ethics Committee (protocol number: 66999417.9.0000.8084).

### Sample Selection

This cross-sectional population study included five-year-old preschool children from two municipal early childhood education schools in São Paulo, SP, Brazil. Data were collected between March and May 2018.

Sample size calculation was based on a previous study of O'Sullivan and Curzon<sup>19</sup> regarding saliva buffering capacities affecting ETW in children. The calculated sample was 22 children per group based on a 95% confidence interval and study power of 80%, using the OpenEpi software (<http://www.openepi.com>).

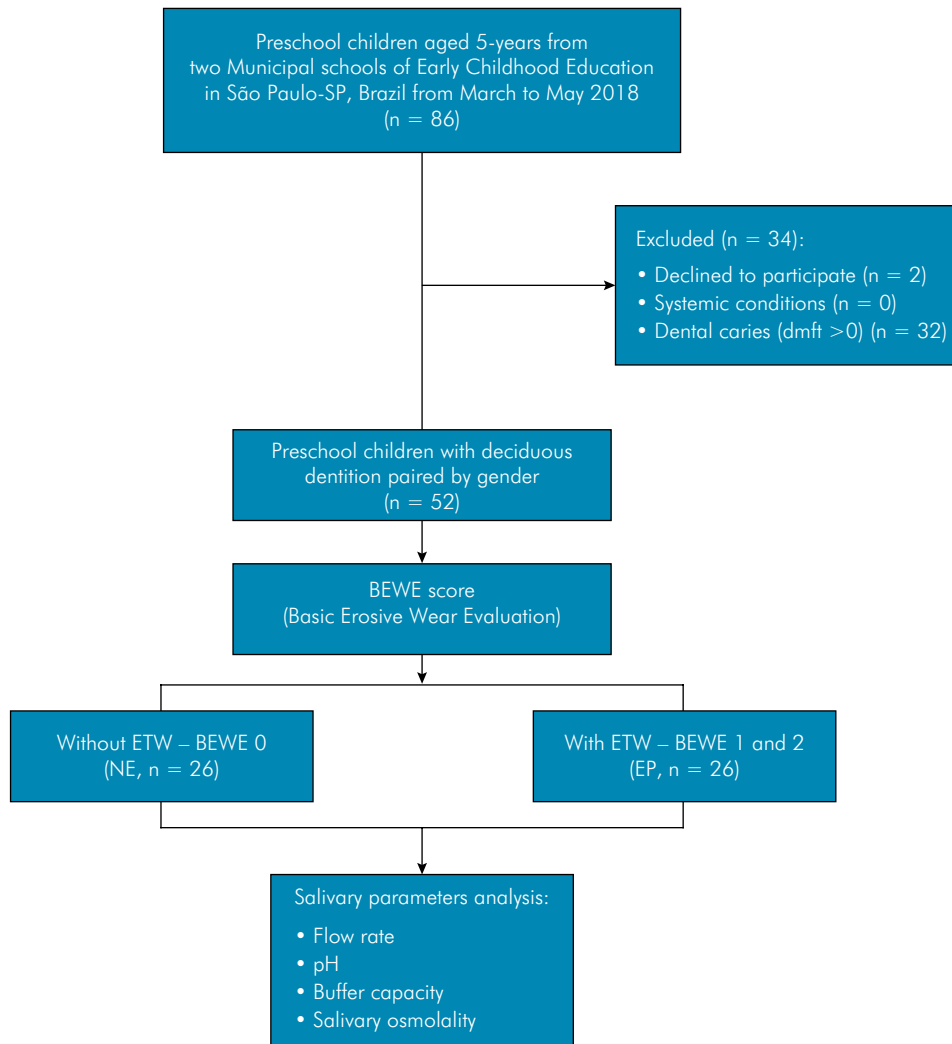
The inclusion criteria were healthy preschool children aged 5 years of both sexes, with complete deciduous dentition and a written informed consent from a parent/guardian. Children who had visible dental caries (dmft > 0), or any type of infection, diabetes, leukemia, and asthmatic bronchitis were excluded from the study. Moreover, children who were taking any drug that could interfere with salivary secretion and those who refused to cooperate with data collection were also excluded.

One examiner (PHC) was trained and calibrated for dental caries assessment using WHO criteria (dmft index) (Kappa = 0.80). Visual examination was performed using a mouth mirror and a ball-ended (WHO) periodontal probe in 15 children aged 5 years who did not participate of the study.

A total of 86 children were invited and 84 children were examined after parental permission to participate in the study. Initially, one calibrated examiner evaluated the children for presence or absence of dental caries. It is known that the presence of several carious lesions and sex can influence salivary parameters. Thus, thirty-two children were excluded due to dental caries (dmft >0) and fifty-two caries-free children were assessed for ETW using the BEWE scoring system, fulfilling the inclusion and exclusion criteria for further salivary parameter analyses. The sample was paired by sex as this is an important factor in ETW prevalence among preschool children (Figure).

### Examiner Calibration for BEWE

One examiner (MMP) was calibrated for the BEWE scoring system by an experienced researcher (ROG). The three-hour lecturer included a theoretical explanation, in which the examiner was introduced to the BEWE scoring system and the details of each score were discussed. Subsequently, the *in lux* calibration was performed by the assessment



**Figure.** Flowchart of the study population.

of 36 photographs of ETW in deciduous teeth, as proposed by Bartlett et al.,<sup>16</sup> followed by the comparison of BEWE scores provided by a reference examiner (AL). Disagreements were discussed until a consensus was reached. The *in vivo* calibration was performed with 15 children who were not involved in the study. Children were examined twice by the researchers MMP and ROG, with a one-week interval between examinations. Disagreements were discussed between the examiners until a consensus was reached. Kappa statistics were calculated to evaluate the intra- and inter-examiner reproducibility (0.80 and 0.71, respectively), which indicated excellent agreement.

### Erosive Tooth Wear Assessment

Before clinical examination, teeth were cleaned with a toothbrush and water to remove dental biofilm. The ETW assessment was performed by one calibrated examiner (MMP) at the participants' schools using portable beds under artificial head LED light and a flat mouth mirror. Teeth were dried using sterilized gauze.

ETW was classified according to the BEWE scoring system. All surfaces of all deciduous teeth were assessed, and the highest score was recorded for each sextant. After the assessments of all the sextants, the sum of the scores was calculated (cumulative score). BEWE classifies ETW according to the following scores:

(0) no erosive tooth wear; (1) initial loss of surface texture; (2) distinct defect, with hard tissue loss <50% of the surface area; and (3) hard tissue loss ≥50% of the surface area.<sup>16</sup> Then, the children were divided into two groups according to ETW prevalence (0 vs ≥ 1): without ETW (BEWE score 0) and with ETW (BEWE scores 1 to 3) (Figure).

### Assessment of Salivary Parameters

Stimulated saliva was collected on another school day, during the same week of clinical examination.

Saliva samples were collected at least two hours after breakfast between 8 am and 10 am to minimize the circadian rhythm. Stimulated saliva was collected after chewing a piece of paraffin (3 cm x 3 cm, 0.7 g).<sup>10</sup> Saliva produced in the first 30 seconds was discarded. The mouth was sanitized with distilled water and the initial saliva was discarded again. During a 5-minute period, the child continued to chew and spit saliva into a 50-mL conical tube. Salivary flow rate was calculated and recorded (mL/min). Saliva was immediately stored on ice in a Styrofoam box and transported to the university laboratory.

A portable digital pH meter (Thermo Scientific Orion STAR A221, Beverly, USA) was used to estimate the baseline salivary pH. The device was calibrated by placing the electrode into three different solutions with known pH values, according to the manufacturer's instructions.

The buffer capacity was determined by titration. Using a glass pipette, 0.2 mL of 0.01 N HCl was added to the 1 mL salivary samples, and the same digital portable pH meter was used. The electrode was washed with distilled water and carefully dried with absorbent paper before the next measure. This process was repeated with the addition of 0.2 mL of HCl, and the pH value was recorded until the value of ≤ 5.5 pH was obtained. The total buffer capacity was obtained, and the three main buffers with pH scale values were represented: up to 7, between 6.9 and 6, and between 5.9 and 5.0.<sup>20</sup>

Immediately after completing these analyses, the samples were centrifuged at 5,000 rpm for 5 min (Hettich® centrifuge, Universal 320R model, Tuttlingen, Germany) and stored at -80°C until analysis. Salivary osmolality was determined after

thawing the samples. Ten microliters of centrifuged saliva was placed on the optical reading disk of the osmometer (VAPRO® Vapor Pressure Osmometer, model 56000; New Instrument, Washington, USA). This device was calibrated by the comparison method using standard solutions (Opti-Mole™ 290 and 1000 mmol/kg Osmolality Standard ELITech Group, WESCOR, Washington, USA).<sup>8</sup>

### Statistical Analysis

Data analyses included descriptive and inferential statistics. All analyzes were performed using SPSS Statistics (IBM, version 20.0) considering a significance level of 5% ( $p < 0.05$ ).

Groups were compared in relation to the studied salivary parameters. Normality assumption and homogeneity of variances were evaluated by Kolmogorov-Smirnov and Levene's tests. Then, Student t-test and chi-square test were performed according to the dependent variable (ETW prevalence) and independent variables (salivary parameters, treated as continuous variables).

Then, logistic regression analysis was performed to determine the magnitude of the association between ETW prevalence and salivary parameters. Variables with  $p < 0.20$  in the univariate analysis were incorporated into the multivariate analysis.<sup>21</sup> Estimates of odds ratios (OR) with their respective 95% confidence intervals (CI) were obtained. The variables "BEWE severity" and "affected sextant" were not incorporated in the regression analysis because they were only applied to the group of children with ETW.

The salivary parameters were compared according to ETW severity (BEWE score 1 versus score 2) in the subgroup of children with ETW.

Receiver operating characteristic (ROC) analyses were performed for salivary osmolality and the occurrence of ETW, and the area under the ROC curve (Az) was calculated. The best cutoff point for salivary osmolality was determined, considering the sum of specificity and sensitivity.

## Results

Figure presents the flowchart of the study.

Preschool children were divided into the following groups: without ETW (n = 26) and with ETW (n = 26). No children had BEWE score 3. Table 1 shows the maximum BEWE score according to the sex of the participants. There was no statistically significance difference between the groups (p > 0.05).

Erosion status (BEWE score) according to sextants in the group with ETW is shown in Table 2. Significant differences in BEWE scores were found for all sextants (p < 0.05). Most children had no ETW (BEWE score 0) (57.7%) in the sextant 5 (lower

anterior teeth). In general, in all sextants, when ETW was diagnosed, BEWE score 1 was more prevalent than score 2. Sextant 2 (upper anterior teeth) was the most affected by BEWE score 2 (30.8%).

Among salivary parameters, only osmolality was statistically different between groups (p < 0.05) (Table 3).

Table 4 shows the results of the logistic regression analysis. In the unadjusted analysis, it was found that children diagnosed with ETW were more likely to have lower salivary flow (OR = 0.079;

**Table 1.** Maximum Basic Erosive Wear Examination score according to sex of the children.

BEWE	Males		Females		Total n (%)	p-value*
	n		n			
0	16		10		26 (50.0)	0.860
1	10		7		17 (32.7)	
2	6		3		9 (17.3)	
3	0		0		0 (0.0)	
Total	32 (61.5%)		20 (38.5%)		52 (100.0)	

\*Chi-square test.

**Table 2.** Erosion status in BEWE score by sextant in the group with ETW.

BEWE score	Sextant, n (%)					
	1	2	3	4	5	6
0	4 (15.4%)	3 (11.5%)	3 (11.5%)	1 (3.8%)	15 (57.7%)	3 (11.5%)
1	22 (84.6%)	15 (57.7%)	23 (88.5%)	24 (92.4%)	8 (30.8%)	23 (88.5%)
2	0 (0%)	8 (30.8%)	0 (0%)	1 (3.8%)	3 (11.5%)	0 (0%)
p-value	< 0.001*	0.015*	< 0.001*	< 0.001*	0.015*	< 0.001*

Chi-square test (\*p < 0.05).

**Table 3.** Comparison of salivary composition between both groups.

Variables	without ETW (mean ± SD)	with ETW (mean ± SD)	p-value
Flow rate, ml/min	0.71 ± 0.35 <sup>A</sup>	0.61 ± 0.43 <sup>A</sup>	0.349
Baseline pH	7.50 ± 0.37 <sup>A</sup>	7.52 ± 0.66 <sup>A</sup>	0.920
Total buffering capacity, mL (0.01 N HCl)	2.41 ± 1.46 <sup>A</sup>	2.15 ± 1.65 <sup>A</sup>	0.547
Buffering capacity according to pH range, mL (0.01 N HCl)			
pH 7.0	0.75 ± 0.53 <sup>A</sup>	0.68 ± 0.60 <sup>A</sup>	0.663
pH 6.9-6.0	1.07 ± 0.71 <sup>A</sup>	0.98 ± 0.79 <sup>A</sup>	0.659
pH 5.9-5.0	0.59 ± 0.34 <sup>A</sup>	0.49 ± 0.35 <sup>A</sup>	0.299
Osmolality (mOsm/kg H <sub>2</sub> O)	232.62 ± 80.14 <sup>A</sup>	187.69 ± 76.18 <sup>B</sup>	0.043*

Different upper-case letters indicate significant difference in the same line (\*p < 0.05, t-Student test).

**Table 4.** Logistic regression analysis to determine the magnitude of the association between ETW and salivary parameters.

Variable	Univariate analysis			Multivariate analysis		
	OR Unadjusted	95%CI	p-value	OR Adjusted	95%CI	p-value
Flow rate, mL/min	0.079	0.013–0.469	0.005*	0.087	0.014–0.533	0.008*
Osmolality (mOsm/kg H <sub>2</sub> O)	0.993	0.985–1.000	0.049*	0.993	0.986–1.001	0.080
pH	1.057	0.373–2.994	0.918	–	–	–
Buffering capacity according to pH range, mL (HCl 0.01N)	0.894	0.624–1.279	0.539	–	–	–
pH 7.0	0.801	0.302–2.126	0.656	–	–	–
pH 6.9–6.0	0.843	0.402–1.770	0.652	–	–	–
pH 5.9–5.0	0.415	0.080–2.148	0.294	–	–	–

OR = odds ratio; CI = confidence interval; \*p < 0.05

**Table 5.** Salivary composition in the group with ETW by ETW severity (BEWE maximum score).

Variables	BEWE 1 (n = 17)	BEWE 2 (n = 9)	p-value
	(mean ± SD)	(mean ± SD)	
Flow rate, ml/min	0.62 ± 0.37 <sup>A</sup>	0.58 ± 0.56 <sup>A</sup>	0.832
Baseline pH	7.40 ± 0.27 <sup>A</sup>	7.74 ± 1.06 <sup>A</sup>	0.225
Total buffering capacity, mL (0.01 N HCl)	0.89 ± 1.40 <sup>A</sup>	1.27 ± 2.18 <sup>A</sup>	0.590
Osmolality (mOsm/kg H <sub>2</sub> O)	214.59 ± 78.24 <sup>A</sup>	136.89 ± 38.10 <sup>B</sup>	0.010*

Different upper-case letters indicate a significant difference in the same line (\*p < 0.05, t-Student test)

**Table 6.** Area under the ROC curve and sensitivity and specificity values for salivary osmolality for the occurrence of ETW and its respective cutoff point.

Variable		
Area	95% CI	p-value
0.645	0.500–0.773	0.068
Cutoff	Sensitivity	Specificity
201	62%	73%

95%CI = 0.013–0.469; p = 0.005) and lower osmolality (OR = 0.993; 95%CI = 0.985–1,000; p = 0.049). In the adjusted analysis, salivary flow remained significantly associated with ETW (OR = 0.087; 95%CI = 0.014–0.533; p = 0.008).

In addition, the salivary parameters were compared in the ETW group according to ETW severity (BEWE maximum score 1 and 2), demonstrating that osmolality levels decreased as ETW severity increased (Table 5).

ROC curve analysis determined a salivary osmolality cutoff point of < 201 for the presence of ETW, with an area under the curve of 0.645 (95%CI 0.500–0.773), a sensitivity of 62.0%, and a specificity of 73.0% (Table 6).

## Discussion

ETW is an oral health problem with high prevalence in children and adolescents<sup>22</sup>, and it is most commonly found at the initial stage when the lesions are restricted to enamel.<sup>22,23</sup> To our knowledge, this is the first study to verify the association between ETW prevalence and salivary parameters (flow, osmolality, pH, and buffering capacity) in preschool children with deciduous dentition.

The null hypotheses were rejected as salivary flow rate was significantly associated with ETW prevalence, and osmolality levels decreased as ETW severity increased in the subgroup of children

with ETW. Literature is limited regarding findings of ETW prevalence and salivary parameters in preschool children, which makes comparing our results challenging.

Saliva plays an important role in the development and progression of ETW<sup>4</sup> since it balances the pH of the oral medium through the exchange of calcium and phosphate ions. As the level of bicarbonate is directly proportional to the salivary flow rate, saliva with a low flow rate presents lower pH and lower buffer capacity.<sup>1,3-5</sup> The buffering capacity of saliva protects the pH from acid induction, neutralizing acids in the oral environment.<sup>24</sup>

Studies have shown that ETW can be related to low salivary flow or/and buffering capacity,<sup>25,26</sup> which is in line with the present study that found that preschool children diagnosed with ETW were more likely to have lower salivary flow. In our study, there were no statistically significant differences in pH and buffering capacity of saliva. In adults, low stimulated salivary flow rate can also be associated with ETW.<sup>27</sup> On the other hand, Shitsuka et al.<sup>10</sup> suggested that children aged 4 to 9 years with ETW present slightly higher salivary pH values, but within the normal physiological range, raising the hypothesis that salivary pH increases with higher ingestion of acidic foods and beverages.

Salivary osmolality can be a good biomarker for the assessment of body fluid status, and is a fast and a non-invasive method to assess hydration status.<sup>28</sup> In the present study, we observed that salivary osmolality (mmol/kg) decreases as ETW severity increases in the subgroup of children with ETW. This could be related to a lower molecular cohesion, leading to increased risk of ETW, with a cutoff point of < 201 for salivary osmolality. The presence of pellicle proteins on the enamel might play an important role in these processes, which could lead to adsorption of pellicle proteins or a change in the composition of the pellicle as salivary osmolality increases or decreases.<sup>29</sup> The protein profile of saliva changes with stimulation by different flavors, so salivary flow can be stimulated mechanically (by chewing) or activated chemically.<sup>30,31</sup> In the present study, saliva was stimulated mechanically.

Like the proteins present in saliva, in the correct adjustments, pellicle proteins are important for protection against ETW.<sup>32,33</sup>

In the present investigation, children with or without ETW were paired by sex. According to Schlueter and Luka,<sup>34</sup> the association between ETW and sex is not clear in children. However, it seems that boys are more affected by dental erosion than girls. The prevalence of ETW increases with the age of the child.<sup>35</sup> In this study, the prevalence of ETW was assessed by the BEWE scoring system in 52 preschool children aged 5 years with deciduous dentition. BEWE is a simple tool that meets most of the formal requirements normally imposed on indices. It is based on a four-point classification system (0-3) that records the most affected surface in a sextant.<sup>16</sup> The cumulative score (mild, moderate, and severe) allows an efficient analysis of ETW.<sup>16,36</sup> It is an important index that can be used in epidemiological studies as well as in monitoring individual cases.<sup>16</sup>

In our study, the most prevalent score in sextant #2 (upper anterior teeth) (30.8%) in the group with ETW was BEWE 2 (ETW with a loss of hard tissue in < 50% of the surface area), which is consistent with the results of the study of Duangthip et al.<sup>37</sup> The upper anterior teeth have higher prevalence of severe ETW, probably because of their early eruption and location, which allow prolonged contact with acidic drinks, increasing the risk for dental erosion. BEWE 0 (no erosive tooth wear) was the most prevalent score in sextant #5 (lower anterior teeth) (57.7%), which is consistent with a previous study<sup>37</sup> showing protection of lower anterior teeth by the tongue.<sup>38</sup>

Over the years, the consumption of acidic foods and drinks has changed in children and adolescents.<sup>12,22,39,40</sup> Acids or chemicals responsible for the etiology of dental erosion can have extrinsic sources (diet and alcohol intake) or intrinsic factors (bulimia, anorexia, and gastrointestinal disorders).<sup>41</sup> This study did not use questionnaires to assess food and beverages consumption in preschool children. All children received a balanced diet from the school and did not consume beverages other than water while at school. However, home dietary habits and

diet quality could influence the results. Moreover, all children evaluated were healthy and no drugs were administered.

Early diagnosis of ETW in children is important for the prevention of irreversible damage to deciduous teeth. Although this study had internal validity for the association of ETW and salivary parameters in preschool children, some limitations should be pointed out: the study design (cross-sectional), the use of a convenience sample, and no assessment of dietary habits of the children. Therefore, future longitudinal studies using the BEWE scoring system and salivary parameters are needed to provide robust evidence on dental erosion in children, evaluating specific proteins or calcium ions in saliva and new treatments with the modification of the salivary pellicle for ETW prevention.<sup>42</sup>

## Conclusions

In conclusion, salivary flow rate was significantly associated with ETW. Lower values of salivary osmolality were observed in the subgroup of preschool children with pronounced erosive lesions with significant loss of tooth structure (BEWE 2).

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