Original Research
Pediatric Dentistry

Salivary profile of children with erosive tooth wear: a transversal study

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Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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https://doi.org/10.1590/1807-3107bor-2020.vol34.0115

Submitted: February 17, 2020
Accepted for publication: July 10, 2020
Last revision: July 31, 2020

Abstract: The aim of the present transversal study was to evaluate the clinical and biochemical salivary parameters of children with and without erosive tooth wear (ETW). The study population was children aged 4 to 9 years. A trained and calibrated examiner (kappa value for intraexaminer reliability = 0.89) classified the children into ETW (n = 24) and control groups (n = 24), and applied the O'Brien index. The salivary flow rate was initially evaluated by stimulated sialometry (paraffin chewing). Afterwards, the collected saliva was submitted to biochemical analyses of pH, uric acid, total buffering capacity, ferric-reducing antioxidant power, reduced glutathione, calcium, and phosphorus. Among the ETW children, 20 (83%) had dental lesions restricted to enamel, and 4 (17%) presented lesions affecting both enamel and dentin. A statistically significant difference between the groups was obtained only for the pH values (t-test; p = 0.004), with averages of 7.31 and 7.56 for the control and the ETW groups, respectively. Considering the parameters evaluated in general, it is suggested that the salivary profile of children with ETW does not differ considerably from that of children without ETW. However, the pH mean value seems to be slightly higher in ETW children, but is still within the normal physiological range.

Keywords: Tooth Erosion; Saliva; Pediatric Dentistry.

Introduction

Erosive tooth wear (ETW) affects dental mineralized tissues, and is considered a complex and multifactorial oral health problem.[1] It is defined as a chemical-mechanical process caused mainly by acid aggression not related to bacteria.[2] The acid challenge may be of either intrinsic (*e.g.*, gastric acid) or extrinsic origin (*e.g.*, dietary habits),[3] and the etiology directly influences the pattern of dental damage.[4]

Overall, ETW prevalence ranges considerably worldwide, recording rates between 0 and 100%, based on studies from different countries and populations.[5] Owing to current lifestyle changes,[6] and the specific features of primary teeth (*e.g.* lower mineral levels in enamel)[7] and saliva,[8,9] children seem to be more prone to ETW today.[10] A systematic review reported a prevalence rate between 0 and 82% of ETW involving the dentin in the primary teeth of children up to 6.5 years, and between 0 and 54% in the permanent teeth of children older than 7 years.[11] Despite

the high prevalence, ETW is found in children mainly in its early stages, which affect only the tooth enamel.[1,13,14]

Since ETW usually leads to loss of the morphology of dental surfaces,[2] these erosive lesions are diagnosed by visual inspection of their morphological features; however, diagnosis is possible only when there is considerable loss of mineralized structure.[15] Although it is very common, ETW becomes a concern only in advanced stages, which affect aesthetics, and lead to functional impairment and dentin hypersensitivity.[6,16] Dentists seek to keep ETW at bay, usually by implementing preemptive measures, which involve using fluorides, recommending oral hygiene practices, and giving dietary counseling.[6]

Saliva is an essential factor for oral health.[17] It protects the teeth against ETW in different ways, mostly by: a) acting directly on acid substances, by diluting, clearing, neutralizing, and buffering acids; b) participating in pellicle formation; c) providing calcium, phosphate, and fluoride for the remineralization process.[18] Although the oral antioxidant system has not yet been fully elucidated, saliva seems to play an important role. This highly complex system is composed of many factors and processes, and may cause cell damage followed by cell death, leading to a decreased formation of dental biofilm, which is also considered a protective mechanical barrier against ETW.[19]

The number of investigations conducted on ETW has increased significantly in recent years; however, too little is known about the salivary profile in children with this condition. In light of this need, the study tested the hypothesis that children with ETW would present alterations in some salivary features, leading to higher susceptibility to ETW. The present study aims at investigating this hypothesis by evaluating clinical and biochemical salivary parameters of children with and without ETW.

Methodology

Study design and ethical issues

This transversal study was previously approved by the Research Ethics Committee of Universidade Cruzeiro do Sul (Protocol #015/2010), and included a convenience sample of 48 children aged 4 to 9 years who attended the Pediatric Dentistry Clinic at Universidade Cruzeiro do Sul (São Paulo, SP, Brazil) in 2010.

All participants met the following inclusion criteria: good general health, no intake of medication that would alter salivary flow/composition, and no inflammatory/infectious oral condition.

Before being submitted to any procedure, the patients received information on the study, together with their parents. The caregivers of those who agreed to participate read and signed the informed consent form.

ETW diagnosis

A trained and calibrated examiner diagnosed ETW, after having previously undergone 2 sessions (4 hours each session) of diagnostic exercises, with 20 clinical images and 20 extracted teeth (varying degrees of wear), for ETW identification. The kappa test for intraexaminer reliability was 0.89.

The examiner ensured correct diagnosis of ETW in the study sample by performing relative isolation (cotton rolls, saliva ejector, and air-jet drying), and using dental mirror #5.

The children were divided into two similar groups, according to the presence of ETW on at least one tooth surface: control (n = 24) and ETW (n = 24) groups. The O'Brien index[20] was used to evaluate ETW severity.

Stimulated sialometry

Sialometry tests were performed on all children from 3 p.m. to 5 p.m. to prevent physiological changes influenced by the circadian rhythm. They were advised to refrain from eating, drinking, or brushing their teeth two hours before the procedures. They remained seated, with their eyes open and heads bent slightly forward during saliva collection.

The children were asked to chew on a tasteless and odorless piece of paraffin (3 cm x 3 cm, 0.7 g) for a total of 6 minutes. The saliva secreted in the first minute was swallowed. That accumulated on the floor of their mouth in the remaining 5 minutes was expectorated into a graduated tube fitted with a funnel. The salivary flow rate was calculated in milliliters per minute (mL/min).

The tubes containing saliva samples were kept in ice for a short period (transportation) and then stored at -80 °C in order to preserve their features.

pH

The pH analysis was performed with an UltraBasic UB-10 digital portable pH meter (Denver Instrument, Bohemia, NY, USA). The device was calibrated by placing the electrode into two different solutions with known pH values, according to the manufacturer's instructions.

Buffering capacity

Buffering capacity was determined by titration with a 0.01N HCl solution.

A 0.2 mL aliquot of 0.01N HCl was added to 1 mL salivary samples, and the same digital portable pH meter was used to check the pH. This process was repeated constantly until the value of ≤ 5.5 pH was obtained.

Uric acid

Uric acid was quantified using a specific biochemical kit (BioClin Quibasa, Belo Horizonte, Brazil).

Uric acid is oxidized by uricase, forming allantoin and H_2O_2, which reacts with 4-aminoantipyrine and 3,5-dichloro-2-hydroxybenzene sulfonate in a peroxidase-catalyzed reaction. The product from this reaction could then be quantified using a spectrophotometer at a 520 nm wavelength.[21]

Ferric-reducing antioxidant power (FRAP)

FRAP was analyzed according to a previous study[21] based on modifications of the method originally described by Benzie and Strain.[22] Changes included replacement of the Fe^{2+}-chelating agent 2,4,6-tri(2-pyridyl)-s-triazine by its analog agent 2,3-bis(2-pyridyl)-pyrazine (DPP).[23] In brief, the reactant mixture for the FRAP assay contained 10 mM DPP (stock solution prepared in 40 mM HCl) and 20 mM $FeCl_3$ in 0.30 M acetate buffering solution (pH 3.6). A 10-20 µL sample was added to 200 µL of the FRAP reactant mixture, together with 40-30 µL distilled water (total volume, 250 µL). Absorbance at 593 nm was recorded for 4 minutes to determine the rate of Fe^{2+}-DPP complex formation, compared with a standard curve.

Reduced glutathione (GSH)

The GSH level in saliva was measured as described by Rahman *et al.*[24] The method is based on the reaction between reduced thiol groups with 5.5′-dithio-*bis*-2- nitrobenzoic acid (DTNB) to form 5-thio-2-nitrobenzoic acid (TNB), which is stoichiometrically detected by absorbance at 412 nm. Purified GSH was used as the standard sample.

Calcium

Calcium was determined according to a method proposed by Ferro and Ham.[25] Salivary calcium was precipitated using chloranilic acid, to yield calcium chloranilate, which was then washed in 50% isopropyl alcohol, and dissolved in a 5% ethylenediaminetetraacetic acid (EDTA) aqueous solution. Calcium phosphate (0.1 mg/mL) was then used as the standard sample to calculate the calcium concentration, using a spectrophotometer at 520 nm.

Phosphorus

Phosphorus was evaluated based on a method described by Fiske and Subarrow.[26] Saliva samples had to be deproteinized by incubation with 1.2 M trichloroacetic acid (TCA), to allow the phosphorus minerals to react with molybdic acid (2.5% ammonium molybdate solution in 10 N sulfuric acid) and form a complex of phosphomolybdic acid. This complex was then reduced by ascorbic acid to form a blue complex, in which the color intensity was proportional to the amount of inorganic phosphorus. The standard curve was determined by spectrophotometry at 720 nm, using a 1 Mmol/mL standard phosphorus solution.

Statistical analysis

The data were tabulated into Microsoft Office Excel™ spreadsheets (Microsoft, USA), and analyzed both descriptively and inferentially using Stata 9.0™ (StataCorp LP, USA) software. The Kolmogorov-Smirnov test was used to check data distribution, and Levene's test, to examine variance equality. The

Table. Averages, standard deviations (±), and effect size (*d*) of each salivary evaluation.

Variable	Group		d	p-value
	Control	Erosive tooth wear		
Salivary Flow Rate (mL/min)	0.52 (± 0.34)	0.60 (± 0.26)	0.26	0.366
Calcium (μg/mL)	183.92 (± 47.88)	195.25 (± 59.63)	0.21	0.472
Phosphorus (mg/mL)	1177.44 (± 261.17)	1141.98 (± 327.10)	0.12	0.680
pH	7.31 (± 0.30)	7.56 (± 0.28)	0.86	0.004*
FRAP (μmol Fe/min mL)	1.44 (± 1.04)	1.44 (± 1.12)	0.0	0.991
Uric Acid (mg/dL)	30.35 (± 16.80)	23.33 (± 11.44)	0.49	0.097
GSH (mM)	0.08 (± 0.05)	0.13 (± 0.12)	0.54	0.121
Total Buffering Capacity (mL HCl 0.01N)	1.23 (± 0.32)	1.41 (± 0.35)	0.54	0.076

*Statistically significant difference (independent t-test, $p < 0.05$).

differences between both groups were analyzed by the independent t-test, and Cohen's *d* effect size was presented for all variables. The level of significance was set at 5%.

The observed power for each parameter was calculated using G*Power 3.197™ software (Universität Kiel, Germany), adopting $\alpha = 0.05$, as follows: salivary flow rate, 23%; calcium, 18%; phosphorus, 11%; pH, 90%; FRAP, 5%; uric acid, 51%; GSH, 58%; total buffering capacity, 57%.

Results

The mean age and standard deviation (±) of ETW and control groups were 7.56 years (±0.29) and 7.31 years (±0.30), respectively. According to the O'Brien index, 20 (83%) ETW children had dental lesions restricted to enamel and 4 (17%) presented with lesions affecting both enamel and dentin.

There was no statistically significant difference between the groups regarding the salivary evaluation, except for the pH mean value, which was slightly higher in the ETW children's group. The data from each salivary evaluation are presented in Table, as well as detailed information on the statistical analyses.

Discussion

The aim of the current study was to evaluate certain salivary parameters used to identify alterations that would make children more likely to present ETW; however, a marked contrast was observed between its findings and the data from the literature, thereby making it difficult to draw definitive conclusions in this respect.

Regarding the characteristics of the sample, some epidemiological studies have encountered a high frequency of ETW in children, but generally in its early stage, i.e., lesions restricted to tooth enamel.[12,13,14] In fact, evaluation by the O'Brien index indicated that a large part of the ETW group had early-stage lesions.[20]

It is important to highlight that the mean pH of both groups can be considered normal (6.5–7.5),[27] but that children with ETW presented a slightly higher value, a result that differs from others in the literature.[19,28] Although the current finding seems to be contradictory, it raises the hypothesis that the basal salivary pH may increase in response to constant exposure to acid agents. To the best of the authors' knowledge, however, there is no evidence to support this.

In line with other studies on children and adolescents,[19,28,29] no difference was seen in the total salivary buffering capacity between the groups. This salivary capacity is based on phosphate, carbonic acid, and bicarbonate systems, which regulate the oral pH.[30] Some authors, however, have asserted that a combination of individual factors (*e.g.*, buffering capacity and salivary flow rate), along with chemical agents, may determine the risk of developing ETW and lesion severity.[1]

Considering the lower salivary flow rate in the deciduous versus permanent dentition,[19] the current

findings corroborate other research results that have found no relationship between the quantity of saliva and ETW.[19,28] On the other hand, a study with adolescents reported a reduced salivary flow rate in individuals with ETW.[29]

Some protective mechanisms are of paramount importance to prevent hard tissue dissolution during an erosive challenge, such as the common ion effect brought about by salivary calcium and phosphate.[15] Furthermore, some peptides and proteins from saliva may bind to the calcium and phosphate on the tooth surface, contributing to the formation of salivary pellicle, which acts as an ion reservoir and partially protects the enamel surface from acidic attack.[31] Although some authors have not found any association between salivary ion content and susceptibility to ETW,[31] certain calcium and phosphorus concentrations have been investigated herein. As in other studies with 12-to-13-year-old children and adolescents,[28,29] no difference was observed.

Shitsuka et al.[19] reported less biofilm in children with ETW than in the controls, but observed no difference in regard to the activity of oxidative stress in saliva. These results corroborate the present findings, which show no differences in the non-enzymatic parameters for FRAP, GSH, and uric acid, all of which have specific antioxidant proprieties.[32] These parameters were singled out for the current study because the FRAP test quantifies both the ability of saliva to chelate and the inactivate metal ions (mainly Fe^{2+} / Fe^{3+}) involved in oxidative stress; GSH is considered a biomarker of oxidative stress; and uric acid acts as a preventive antioxidant and a scavenger of free radicals that cause oxidative damage.[21]

Given the lack of sufficient evidence supporting the salivary profile in children with ETW, the results of this study are still a relevant contribution to the literature; however, they should be interpreted with caution. In regard to study limitations, some issues should be pointed out. First, a priori sample size estimation was not considered, which may have influenced the results, especially the likelihood of failing to detect true differences between the groups (type-2 error). Taking into account that the statistical power and *P*-values depend both on the size of the effect and the sample size,[33] the convenience sample used herein probably did not provide sufficient power to detect statistical differences between both groups, as noted by the (post hoc) power calculated for each parameter.

The cut-off point used to allocate the children into the groups (i.e., at least one surface presenting ETW) is another potential study limitation, since it might have made the whole sample more homogeneous, and less prone to presenting the salivary parameter discrepancies between both groups. Therefore, more research focusing on the classification of children according to ETW stages is needed, with more in-depth information on the grouping adopted.

Conclusion

Within the limitations of this study, and considering the parameters evaluated, the findings seem to suggest that the salivary profile of children with ETW does not differ considerably from that of children without ETW. However, the pH mean value seems slightly higher in ETW children, but is still within the normal physiological range.

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