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Unstimulated and stimulated salivary characteristics of 12–13-year-old schoolchildren with and without dental erosion

Ping Wang [a], *Yan Zhou* [b], *Yuan Hang Zhu* [c], *Huan Cai Lin* [b,*]

[a] *Department of Stomatology, the Third Affiliated Hospital, Sun Yat-sen University, 600 Tian He Road, Guangzhou, Guangdong, China*
[b] *Department of Dental Public Health, Guanghua School of Stomatology, Sun Yat-sen University, 56 Ling Yuan Xi Road, Guangzhou, Guangdong, China*
[c] *Department of Laboratory, the Third Affiliated Hospital, Sun Yat-sen University, 600 Tian He Road, Guangzhou, Guangdong, China*

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ABSTRACT

Objective: To evaluate unstimulated and stimulated salivary characteristics of 12–13-year-old schoolchildren with and without dental erosion.
Design: The subjects were sixty schoolchildren from 12–13 years old (30 boys and 30 girls) with dental erosion and sixty age- and sex-matched controls. Unstimulated and stimulated whole saliva were collected. Flow rate, pH level, buffering capacity, bicarbonate, buffer base, calcium, phosphorus and urea concentrations of whole saliva were measured. All data were analysed using SPSS 13.0.
Results: The flow rate, pH, bicarbonate, buffer base, calcium, phosphorus, and urea of unstimulated and stimulated saliva did not differ significantly between the dental erosion group and the control group ($P > 0.05$). The stimulated salivary buffering capacity did not vary between the two groups (Fisher's exact test, $P > 0.05$).
Conclusion: The salivary characteristics are similar amongst 12–13-year-old schoolchildren with and without dental erosion in Southern China.

1. Introduction

Dental erosion is commonly defined as chemical wear of dental hard tissues without bacterial involvement.[1] Its aetiology is multifactorial; its causes, which are classified as intrinsic or extrinsic acids, include gastric acid, carbonated drinks, sports drinks, fruit juices, wine, industrial acid fumes, acidic medications, such as aspirin, and improperly chlorinated swimming pools.[2]

The biological factors related to dental erosion may involve the properties and characteristics of saliva, acquired dental pellicle, tooth structure and surrounding soft tissues.[3] Saliva has been considered the most important biological factor preventing dental erosion.[4] It is composed of a variety of electrolytes, immunoglobulins, proteins, enzymes, mucins and nitrogenous products.[5] Dawes noted that simulating the flow of saliva altered its composition. Perhaps of greatest importance is the increase in the concentration of bicarbonate, which increases progressively with the duration of stimulation.[6] Saliva protects against dental erosion due to its many properties, which include dilution and clearance of an erosive agent from the mouth; neutralization and buffering of dietary acids; formation of the acquired pellicle that protects the enamel surface from demineralization by dietary acids; and provision of calcium, phosphate and possibly fluoride, which are necessary for remineralization.[7]

* *Corresponding author*. Tel.: +86 20 8386 2560; fax: +86 20 8382 2807.
E-mail address: lin_hc@163.net (H.C. Lin).
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Previous studies have evaluated the normal values and variations of salivary characteristics in children.[8–11] Some of the studies that investigated the salivary factors, which may influence dental erosion, produced conflicting results concerning a possible relation between salivary characteristics and dental erosion.[12–18] Several case-control studies explored the relationship between saliva and dental erosion in the population with systemic diseases, such as gastro-oesophageal reflex,[19,20] asthma,[21] bulimia nervosa[22,23] and chronic renal failure.[24]

The objective of this case-control study was to compare unstimulated and stimulated salivary characteristics in 12–13-year-old schoolchildren with and without dental erosion in Guangzhou, Southern China, to determine whether salivary factors affect dental erosion in our study individuals.

2. Materials and methods

2.1. Participants

In 2008, we carried out a cross-sectional survey on dental erosion across a population of 1499 12–13-year-old schoolchildren from 10 schools in five districts of Guangzhou, the capital city of Guangdong Province in Southern China. The prevalence of dental erosion in our survey was found to be 27.3%.[25] Subjects who met the salivary test requirements were enrolled, and all who participated in the study did so willingly. Sixty schoolchildren (30 males and 30 females) with at least one tooth surface showing loss of enamel surface contour and dental erosion identified as Grade 2 severity on the O'Sullivan index[26] were selected as the case group. Sixty age- and sex-matched children free from erosion were selected as the control group. All schoolchildren enrolled were without systemic diseases, active caries (clinically visible caries or two or more surfaces restored with conventional restorative materials)[14] or having taken medicine within the last two weeks.

The study protocol was approved by the Research Ethics Committee of the Guanghua School of Stomatology, Sun Yat-sen University. The children and their parents or guardians signed an informed-consent letter before participating in the investigation.

2.2. Saliva collection

Saliva was always collected between 16:00 and 17:00 h in a quiet, isolated room. All subjects refrained from eating, drinking and oral hygiene for at least 1 h prior to saliva collection. Prior to collection, the subjects were fully informed of the process of saliva collection.

The procedure of unstimulated saliva collection was as follows: first, the children were asked to swallow their saliva. Then, they were asked to resist swallowing and to spit into an ice-cooled graduated test tube approximately every 30 s for 10 min. When the collection was over, the amount of saliva (ignoring the foam) was measured to an accuracy of 0.1 ml and recorded. A stimulated saliva sample was collected from each subject after the unstimulated saliva collection. The subjects were seated and relaxed for several minutes prior to saliva collection. A 1-g piece of unflavoured paraffin wax (Orion Diagnostica, Espoo, Finland) was chewed for 30 s and the saliva produced was initially swallowed. Each child was then asked to chew at a regular rate of approximately 60 cycle/min and expectorate into a second ice-cooled graduated test tube for 5 min. Again, the volume of saliva collected was read and recorded.

The salivary samples were sealed immediately and transported to the laboratory within 30 min using an icebox. A drop of stimulated saliva was used to determine the stimulated salivary buffering capacity, and a small aliquot from unstimulated and stimulated saliva was used to determine pH, bicarbonate and buffer base concentration immediately in order to avoid any time-related pH changes or CO_2 loss. Aliquots of the residual saliva were collected in Eppendorf Micro Centrifuge Tubes and frozen (−80 °C) for further analyses.

2.3. Saliva analysis

The pH, bicarbonate and buffer base concentration in the unstimulated and stimulated whole saliva of children with or without dental erosion were measured at the Department of Laboratory, the Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, as were the calcium, phosphorus, and urea concentrations. Saliva samples were thawed and centrifuged for 10 min at $3000 \times g$ before the calcium, phosphorus, and urea analysis. In addition, the buffering capacity of the stimulated saliva of both groups was tested using a commercial Dentobuff Strip Kit (Orion Diagnostica, Espoo, Finland) at the Medical Research Center of the Third Affiliated Hospital, Sun Yat-sen University.

2.3.1. Assessment of pH level, bicarbonate and buffer base concentration

The pH, bicarbonate and buffer base concentrations were measured using a blood gas analyser (AVL-COMPACT I, AVL Medical Instr. AG, Schaffhausen, Switzerland). During the test at the lab, all samples were kept on ice, and the measurements were completed within 30 min.

2.3.2. Assessment of buffering capacity of the stimulated saliva

The buffering capacity of the stimulated saliva was measured using the Dentobuff Strip Kit. According to the product instructions, the Dentobuff was intended for use as a strip method to estimate stimulated salivary buffering capacity. The colour changed on the strip was compared with the manufacturer's Colour Chart and read as low, medium or high.

2.3.3. Assessment of calcium, phosphorus and urea concentration

Analyses of calcium, phosphorus and urea were carried out in one session using an automated clinical biochemistry analyser (HITACHI 7180, Tokyo, Japan).[15,24] The test principles for the calcium, phosphorous and urea content were based on the o-cresolphthalein complexone (oCPC) colorimetric assay,[27] ultraviolet method[24] and ultraviolet-glutamate dehydrogenase (UV-GLDH) method,[28] respectively.