

Erosive effect of an antihistamine liquid formulation on bovine teeth: influence of exposure time

Viviane Santos da Silva Pierro¹, Beatriz Romeiro Furtado², Michele Villardi³,
Lúcio Mendes Cabral⁴, Eduardo Moreira Silva⁵, Lucianne Cople Maia⁶

¹DDS, MS, Graduate student, Department of Pediatric Dentistry and Orthodontics, Dental School, Federal University of Rio de Janeiro, Brazil

²DDS, Undergraduate student, Department of Pediatric Dentistry and Orthodontics, Dental School, Federal University of Rio de Janeiro, Brazil

³DDS, Undergraduate student, Department of Medicaments, Pharmacy School, Federal University of Rio de Janeiro, Brazil

⁴MSc, PhD, Pharmacist, Associate Professor, Department of Medicaments, Pharmacy School, Federal University of Rio de Janeiro, Brazil

⁵DDS, MS, PhD, Associate Professor, Department of Restorative Dentistry, Dental School, Federal Fluminense University, Brazil

⁶DDS, MS, PhD, Associate professor, Department of Pediatric Dentistry and Orthodontics, School of Dentistry, Federal University of Rio de Janeiro, Brazil

Abstract

Aim: To evaluate, *in vitro*, the effect of an oral antihistamine liquid formulation on roughness and topography of bovine enamel and the influence of exposure time on its erosive effect. **Methods:** Forty-one bovine enamel blocks were prepared leaving an exposed window of 0.8 mm². Thirty-nine blocks were divided into three treatment groups according to media immersion: antihistamine formulation (Histamin[®]), 0.6% citric acid (positive control), and distilled water (negative control). Before immersion of the samples, pH, titratable acidity, calcium, phosphate and fluoride contents of all media were verified. Enamel roughness was evaluated at baseline, and after 5, 15, and 30 min of immersion (9 samples *per* group). Two specimens from each group and exposure time, and 2 additional specimens representing baseline, were analyzed by scanning electron microscopy (SEM). Data were analyzed by the Kruskal-Wallis test, and the Mann-Whitney test using the Bonferroni correction ($\alpha=0.017$). **Results:** Specimens immersed in citric acid showed the highest roughness ($P<0.001$). SEM images showed a progressive erosion pattern in samples immersed in citric acid and in antihistamine formulation. **Conclusions:** The antihistamine liquid formulation did not promote significant alterations of enamel roughness. Nevertheless, SEM demonstrated that the antihistamine eroded bovine enamel, and the erosion pattern was influenced by exposure time.

Keywords: tooth erosion, pharmaceutical preparations, hydrogen-ion concentration, child.

Introduction

Dental erosion is the irreversible loss of dental hard tissue due to a chemical process of acid dissolution that does not involve bacterial plaque acid, and is not directly associated with mechanical or traumatic factors¹. The etiology of erosion is related to different behavioral, biological and chemical factors and it can have extrinsic or intrinsic causes. Extrinsic erosion is the result of exogenous acids, and dietary acids are, undoubtedly, its main causative factors. The most frequently consumed potentially damaging beverages are fruit juices and soft drinks². However, several cases of tooth erosion have been attributed to oral administration of medicines³.

Medicines usually have low endogenous pH, high titratable acidity, and absence or low concentrations of ions including those of calcium, fluoride, and phosphate in their composition. The risk of dental erosion is increased when medications are used for treatment of chronic diseases with a high frequency of

Received for publication: October 13, 2009

Accepted: March 25, 2010

Correspondence to:

Lucianne Cople Maia

Rua Gastão Gonçalves, 47/501 - Santa Rosa

24.240-030 - Niterói, RJ, Brazil

E-mail: rorefa@terra.com.br

ingestion (two or more times per day), at bedtime, or when they have side effects such as reduction of salivary flow rate, which happens with antihistamines⁴⁻⁶.

Although the contact of a solution with the teeth during intake is usually not as long as when the liquid is rinsed³, at least one of the daily medication doses are designated to be given at bedtime. Additionally, liquid medicines usually take a longer time to clear from the mouth compared to tablets and capsules⁷.

The pH of liquid oral medicines may be formulated to optimize efficacy and patient acceptability. The solubility of weak acids and bases is pH dependent, and acidic preparations are often necessary for drug dispersion. Additionally, these acidic medicines often taste pleasanter, which may enhance patient compliance, especially children⁸.

Previous studies have already pointed out the erosive potential of liquid medicines or soluble tablets^{4-5,8-12}, but few of them evaluated the effect of oral medication on tooth surface^{4,9-10,12}, and only one study investigated the effect of exposure time on the development of dental erosion⁹. Therefore, this study aimed to evaluate, *in vitro*, the effect of an oral antihistamine liquid formulation on roughness and topography of bovine enamel and the influence of exposure time on its erosive effect, if any.

Material and methods

Medicine selection, pH analysis, titratable acidity and mineral content of the tested media

The most widely distributed antihistamine liquid formulation (Histamin[®], Neo Química, Anápolis, GO, Brazil) by the pharmacies of public Pediatric Hospitals and other Healthcare Services in the city of Rio de Janeiro, Brazil, was chosen for this study because of its possible impact on the oral health of children who usually seek for medical assistance at these facilities. Negative (distilled water) and positive (0.6% citric acid) controls were also tested.

All the 3 tested media were analyzed in triplicate with regard to pH, titratable acidity, calcium, phosphate and fluoride contents. The pH was measured with a calibrated pH meter (Quimis Q-400MT, Diadema, SP, Brazil), which was also used to determine titratable acidity by adding increasing quantities of 0.1 N sodium hydroxide (NaOH) solution to 25 mL of the medicine and controls, followed by agitation and equilibration (2 min) for further pH measurements. A correction factor of 0.95 was obtained by factorizing 0.1 N NaOH solution with potassium biphthalate standard. The total volume of 0.1 N NaOH solution required to neutralize medicines and controls (raise their pH to 7.0) multiplied by the correction factor of 0.95 corresponded to the titratable acidity value. All the media were used in an undiluted form.

Phosphate and fluoride contents of the antihistamine formulation and controls were analyzed by ion chromatography¹³, while calcium content was determined by flame atomic absorption spectrophotometry¹⁴.

Preparation of bovine enamel specimens

Forty-one enamel blocks (3 x 3 x 3 mm) were prepared

from bovine incisors stored in a 2% formaldehyde solution (pH 7.0) at room temperature. Crowns were sectioned from the roots and enamel blocks were obtained with water-cooled diamond double-faced disk at low-speed.

All enamel blocks were then embedded in acrylic resin using PVC rings as moulds with the labial surfaces facing toward the ring base. After resin acrylic polymerization, the labial enamel of samples were wet ground using 600, 800 and 1200-grit abrasive discs (3M, Sumaré, Brazil) for 10 min each and polished using felt discs (Arotec Ind.& Com. Ltda; São Paulo, Brazil) and 1 and 0.3 μm aluminum oxide suspension (South Bay Technology Inc.; San Clemente, USA) in a water-cooled grinding machine (Panambra DPU-10, Struers; Copenhagen, Denmark) to produce a flat surface.

After the polishing procedure, samples were cleaned with an ultrasonic device and viewed under an optical microscope (Aus Jena, model 444181, with a 40 objective; Astro Optics Division, Montpelier, USA) in order to confirm that the surfaces were flat, polished, and free of irregularities that could interfere with roughness evaluation.

Red nail polish (two coats, 24 h drying) was then placed over the enamel blocks, except for a 0.8 mm² window of exposed enamel.

Treatment groups

Thirty-nine specimens were randomly divided into three treatment groups, as follows: antihistamine liquid formulation (Histamin[®], Neo Química, Anápolis, GO, Brazil), 0.6% citric acid (Crystal Pharm, Niterói, RJ, Brazil) – positive control, and distilled water – negative control. The specimens were immersed in 100 mL of each solution for 5, 15, and 30 min at 37 °C and maintained upon agitation during the experiment.

Two additional specimens were set aside and kept in humid environment at 37°C to represent baseline on further SEM analysis.

Roughness Evaluation

All measurements of enamel loss were made using a surface roughness tester (Surftest SJ 201, Mitutoyo Co., Kawasaki, Japan) to determine surface roughness (R_a – μm) due to media exposure.

Before roughness measurements, all specimens were ultrasonicated both at baseline and after the experimental periods. Three roughness measurements spaced at 60° were recorded for each specimen (cut-off length of 0.25 mm) at baseline and after each treatment period (5, 15 and 30 min). Therefore, a mean roughness value was obtained for each sample according to treatment and exposure time (n=9), for both experimental and control groups.

Scanning Electronic Microscopy (SEM) Analysis

SEM analysis was performed to assess the topography of enamel surface at baseline and after each treatment period for the three tested media. Two enamel specimens from each treatment group according to exposure time were prepared for SEM analysis. The two specimens were mounted on aluminum stubs, fully-dried, sputter-coated with a thin layer

of gold-palladium and examined with a JEOL JSM-35 scanning electronic microscope (Tokyo, Japan) with an acceleration voltage of 15kV. SEM micrographs were made at 500x and 2000x magnification.

Statistical Analysis

Statistical analysis was performed using SPSS software version 11.0 (SPSS Inc., Chicago, USA). After verifying that the roughness data presented a non-normal distribution for all treatment groups, Kruskal-Wallis test was used for comparison among the three treatment groups, and then the Mann-Whitney test using Bonferroni correction ($\alpha = .017$) was employed for group comparisons.

Results

The chemical parameters of the media tested in the present study are shown in Table 1.

The results of surface roughness are shown in Figure 1 and Table 2. Kruskal-Wallis test detected significant difference for media ($P < 0.001$). Mann-Whitney test with Bonferroni correction showed that specimens immersed in citric acid presented the highest roughness values ($P < .001$) at all time periods, followed by the antihistamine liquid formulation and distilled water, without significance between them after the 5-min ($P = .66$), 15-min ($P = .60$) and 30-min ($P = .66$) exposures.

Qualitative analysis of SEM micrographs showed that specimens immersed in citric acid presented the most severe erosion pattern with time (Figure 2), followed by those immersed in antihistamine liquid formulation (Figure 3). It seemed that specimens immersed in water did not suffer erosion irrespective of exposure time (Figure 4).

Discussion

Erosive damage to the permanent teeth in early childhood may compromise the dentition for the child's entire lifetime and this will certainly require expensive dental treatments in adult life¹⁵. However, individual susceptibility should be taken into account due to structural variations in enamel. Bovine teeth were used in the present study based on a morphological investigation on progression of enamel erosion, and it has been shown that the surface ultrastructure of erosive lesions in prismatic human enamel did not differ from that observed in bovine tooth specimens¹⁶.

Previous *in vitro* studies have already shown that oral

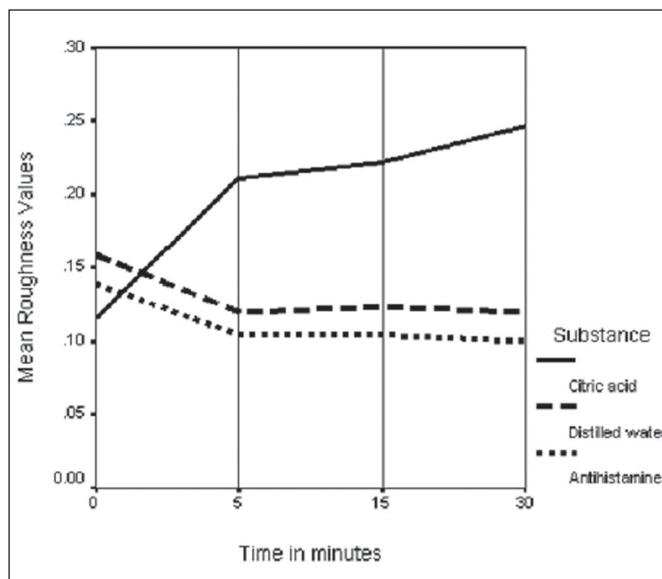


Fig. 1 – Mean roughness values of bovine enamel specimens according to media and exposure time

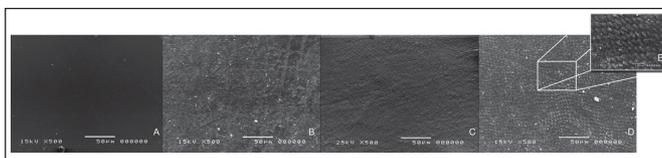


Fig. 2 – Representative SEM micrographs of bovine enamel at baseline (A) and after immersion in 0.6% citric acid (positive control) for 5 min (B), 15 min (C) and 30 min (D). Note extensive eroded areas at higher magnification (x2000) (E).



Fig. 3 – Representative SEM micrographs of bovine enamel at baseline (A) and after immersion in antihistamine liquid formulation (Histamin®) for 5 min (B), 15 min (C) and 30 min (D). Note the presence of eroded areas at higher magnification (x2000) (E).

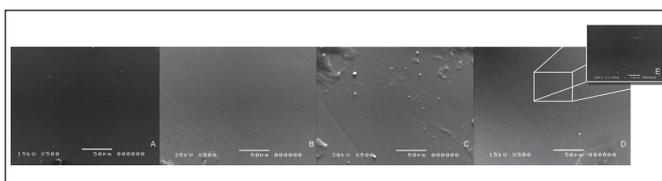


Fig. 4 – Representative SEM micrographs of bovine enamel at baseline (A) and after immersion in distilled water (negative control) for 5 min (B), 15 min (C) and 30 min (D). Note absence of eroded areas at higher magnification (x2000) (E).

Table 1 – Chemical parameters of the antihistamine formulation and controls tested in the present study

Characteristics	Antihistamine formulation	Positive Control	Negative Control
Commercial name	Histamin®	Citric acid	Distilled water
Pharmaceutical name	Dexchlorpheniramine	Citric acid	Distilled water
Batch number	75974	—	—
Expiration date	05/2009	02/12/2008	—
pH	5.1	2.0	5.6
Titrate acidity (volume of 0.1 N NaOH – ml)	1.6	19	0.1
Calcium (mg/L)	1.2	1.4	0.1
Phosphate (mg/L)	< 0.1	3.61	< 0.1
Fluoride (mg/L)	0.99	0.23	< 0.1

medicines could be related to dental erosion^{4-5,8-12}. Additionally, Johansson *et al.*¹⁷, in an *in vivo* investigation with Saudi children, pointed out that high intake of acidic drinks and fruits, upper respiratory tract problems and frequent medications may constitute possible etiological and/or aggravating factors for severe dental erosion.

The medicine selected for this study is an antihistamine liquid formulation extensively handled in public Pediatric Hospitals and Healthcare Services of Rio de Janeiro city and, therefore, consumed by a significant number of children that do not have access to private medical assistance. This antihistamine liquid formulation, like others, is most commonly used for treatment of respiratory allergies, which are usually present as a chronic health problem. As this medication is used chronically by allergic children, it is important to study its chemical properties to verify their role in children's dental health.

Factors mostly related with the ingested substances *per se* could modify erosion patterns. pH and dissociation constants of the acids allied to the general chemical composition of the solutions may modify the degree of enamel dissolution¹⁶. In the present study, the specimens immersed in citric acid (positive control), presented remarkable changes in enamel surfaces than those immersed in antihistamine liquid formulation and distilled water (Figures 2, 3 and 4). At a first glance, this finding could be related to the low pH of this acid (Table 1). However, it is well-known that the pH gives only the initial concentration of H⁺ ions, and does not represent the presence of undissociated acid in the medium^{15,18-20}. Valinoti *et al.*²¹ using two acidic medicines with pH close to the same value (Claritin/antihistamine – 2.57 and Dimetapp/elixir – 2.51) showed, under SEM, that Claritin/antihistamine caused more surface alterations on some resin-based composites. According to these authors, the dissimilar results of these medicines were due to the differences in the titratable acidity between the medicines, 41.83 and 36.31 mL NaOH, respectively. Based on this, it is clear that the higher titratable acidity of citric acid in the present study have contributed more than pH to the changes produced in the enamel surfaces. In fact, titratable acidity can be claimed as a more accurate measure of the total acid content present in substances, and calcium-chelating properties may greatly enhance the erosive potential of beverages^{15,18-20}. The studied antihistamine liquid formulation presented a pH slightly lower than distilled water, but it showed higher buffering capacity and ability to resist pH

changes than distilled water because of its amount of neutralizing agent was tenfold greater than that of water. This probably explains the different patterns of erosion found in SEM images, that is, eroded areas were present in antihistamine (pH 5.1; 1.6 mL NaOH) treated enamel specimens, while no erosive pattern was verified in the samples treated with distilled water (pH 5.6; 0.1 mL NaOH).

Ions like calcium, phosphate and fluoride have a protective effect against erosion¹⁵. However, despite the presence of fluoride and calcium in the antihistamine liquid formulation, and of fluoride, calcium and phosphate in citric acid (Table 1), their ionic concentrations were not high enough to prevent the erosive effect viewed in the enamel surfaces (Figures 2 and 3) on teeth treated with those substances. Here, again, it seems obvious that the high titratable acidity overcame the probable positive effects of ions F³⁻, Ca⁺⁺ and PO₄³⁻ on preventing enamel erosion.

The etiology of dental erosion can also be influenced by biological and behavioral factors, unusual eating, drinking and swallowing habits, for example holding an acid beverage in the mouth before swallowing, increase the contact time of an acid substance with the teeth and thus increase the risk of erosion¹⁵. Furthermore, it should be emphasized that some oral medicines are usually given at bedtime without subsequent toothbrushing or water rinse, and oral clearance is usually also compromised during sleep. Therefore, it seems reasonable to evaluate how exposure time could influence erosive dental patterns. The choice for 5-, 15- and 30-min exposure times were intentionally proposed because they seem to be less aggressive than the time intervals proposed by Babu *et al.*⁹. On this matter, the SEM results of the present study differed from those of Babu *et al.*⁹ because our images showed that severity of erosion's increased with exposure time for both the positive control and the tested antihistamine liquid formulation, while those authors found more severe lesions after a 10-min than an 8-hour immersion period. This could probably be explained by the great difference between these time periods, which could have been enough to promote saturation of the liquid medium.

Quantitative methods are usually employed for determination of erosion severity along with SEM evaluation^{15,22} and roughness analysis has already been used to evaluate the effect of acidic medicines, under pH-cycling conditions, on the surface degradation of composite resins²¹. However, in this study, roughness measurement was not sensitive enough to detect the alterations shown by SEM

Table 2 – Descriptive statistics of roughness values (Ra – μm) by treatment groups and time intervals.

Descriptive statistics	Antihistamine formulation(n=9)				0.6% Citric Acid (n=9)*				Distilled Water (n=9)			
	0	5min	15min	30min	0	5min	15min	30min	0	5min	15min	30min
Mean	0.14	0.10	0.10	0.10	0.12	0.21	0.22	0.25	0.16	0.12	0.12	0.12
Standard deviation	0.07	0.02	0.01	0.02	0.02	0.06	0.04	0.04	0.05	0.02	0.01	0.02
Minimum	0.06	0.08	0.09	0.07	0.08	0.16	0.18	0.19	0.08	0.10	0.11	0.08
1st quartile	0.09	0.09	0.09	0.09	0.10	0.16	0.19	0.21	0.13	0.11	0.11	0.11
Median	0.11	0.10	0.10	0.10	0.12	0.19	0.21	0.25	0.16	0.11	0.12	0.12
3rd quartile	0.18	0.12	0.12	0.12	0.13	0.26	0.26	0.28	0.19	0.14	0.14	0.14
Maximum	0.30	0.13	0.12	0.12	0.14	0.32	0.30	0.30	0.23	0.14	0.14	0.15

*P<.001 (Mann-Whitney test with Bonferroni correction).

analysis in enamel immersed in antihistamine liquid formulation. This was a limitation of the present study with regard to the quantification of mineral loss. Further studies would benefit from combining SEM evaluation with techniques that enable ultrastructural mineral loss quantification.

The difficulty in reproducing the clinical situation in *in vitro* studies must be borne in mind. This is due to complexity of the oral environment. The absence of salivary pellicle and buffering by saliva may overestimate the *in vivo* occurrence of erosive demineralization⁴. However, antihistamine-containing medicines are known to reduce salivary secretion and it is important to alert for their erosive potential even, based mainly on *in vitro* studies⁵. In the present study, enamel specimens treated with the antihistamine liquid formulation showed erosion in SEM images when compared to distilled water, and their erosive pattern increased with increasing exposure time. Further studies testing low-pH antihistamine liquid formulations should be encouraged because allergic children make regular use of these medicines and could present dental erosion as an undesirable effect of oral medication.

Based on the experimental conditions of this study, the following conclusions can be made: among the tested media, only 0.6% citric acid produced a significant increase in enamel roughness. The SEM analysis showed that antihistamine liquid formulation and 0.6% citric acid were capable of producing erosion in enamel and was influenced by exposure time.

Acknowledgements

The authors are grateful to Prof. Paulo Barrocas and Letícia Alves da Silva for their support in the phosphate and fluoride content analysis at ENSP/FIOCRUZ (Escola Nacional de Saúde Pública/Fundação Oswaldo Cruz). The authors also would like to thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the research grant (302382/2009-7), and FAPERJ (Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro) (E-26/110.486/2010) for the financial support.

References

- O'Sullivan E, Milosevic A. UK National Clinical Guidelines in Paediatric Dentistry: diagnosis, prevention and management of dental erosion. *Int J Paediatr Dent.* 2008; 18: 29-38.
- Magalhães AC, Wiegand A, Rios D, Honório HM, Buzalaf MAR. Insights into preventive measures for dental erosion. *J Appl Oral Sci.* 2009; 17: 75-86.
- Moss SJ. Dental Erosion. *Int Dent J.* 1998; 48: 529-39.
- Costa CC, Almeida ICS, Costa Filho LC. Erosive effect of an antihistamine-containing syrup on primary enamel and its reduction by fluoride dentifrice. *Int J Paediatr Dent.* 2006; 16: 174-80.
- Pierro VSS, Abdelnur JP, Maia LC, Trugo LC. Free sugar concentration and pH of paediatric medicines in Brazil. *Community Dent Health.* 2005; 22: 180-3.
- Pierro VSS, Barcelos R, Maia LC, Silva AN. Pediatricians' perception about the use of antibiotics and dental caries – a preliminary study. *J Public Health Dent.* 2004; 64: 244-8.
- Maguire A, Baqir W. Prevalence of long-term use of medicines with prolonged oral clearance in the elderly: a survey in north east England. *Br Dent J.* 2000; 189: 267-72.
- Nunn JH, Ng SKF, Sharkey I, Coulthard M. The dental implications of chronic use of acidic medicines in medically compromised children. *Pharm World Sci.* 2001; 23: 118-9.
- Babu KLG, Rai K, Hedge AM. Pediatric liquid medicaments – do they erode the teeth surface? An *in vitro* study: part I. *J Clin Pediatr Dent.* 2008; 32: 189-94.
- Costa CC, Almeida ICS, Costa Filho LC, Oshima HMS. Morphology evaluation of primary enamel exposed to antihistamine and fluoride dentifrice – an *in vitro* study. *Gen Dent.* 2006; 54: 21-7.
- Maguire A, Baqir W, Nunn JH. Are sugars- free medicines more erosive than sugars-containing medicines? An *in vitro* study of paediatric medicines with prolonged oral clearance used regularly and long-term by children. *Int J Paediatr Dent.* 2007; 17: 231-8.
- McNally LM, Barbour ME, O'Sullivan DJ, Jagger DC. An *in vitro* investigation of the effect of some analgesics on human enamel. *J Oral Rehabil.* 2006; 33: 529-32.
- Tanaka M, Margolis HC. Release of mineral ions in dental plaque following acid production. *Arch Oral Biol.* 1999; 44: 253-8.
- Udoh AP. Atomic absorption spectrometric determination of calcium and other metallic elements in some animal protein sources. *Talanta.* 2000; 52: 749-54.
- Lussi A, Kohler N, Zero D, Schaffner M, Megert B. A comparison of the erosive potential of different beverages in primary and permanent teeth using an *in vitro* model. *Eur J Oral Sci.* 2000; 108: 110-4.
- Meurman JH, Frank RM. Progression and surface ultrastructure of *in vitro* caused erosive lesions in human and bovine enamel. *Caries Res.* 1991; 25: 81-7.
- Johansson A-K, Sorvari R, Birkhed D, Meurman JH. Dental erosion in deciduous teeth – an *in vivo* and *in vitro* study. *J Dent.* 2001; 29: 333-40.
- Cairns AM, Watson M, Creanor SL, Fove RH. The pH and titratable acidity of a range of diluting drinks and their potential effect on dental erosion. *J Dent.* 2002; 30: 313-7.
- Grenby TH, Phillips A, Desai T, Mistry M. Laboratory studies of the dental properties of soft drinks. *Br J Nutr.* 1989; 62: 451-64.
- Larsen MJ, Nyvad B. Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate. *Caries Res.* 1999; 33: 81-7.
- Valinoti AC, Neves BG, da Silva EM, Maia LC. Surface degradation of composite resins by acidic medicines and pH-cycling. *J Appl Oral Sci.* 2008; 16: 257-65.
- Barbour ME, Rees JS. The laboratory assessment of enamel erosion: a review. *J Dent.* 2004; 32: 591-602.