Original Article

Drink to Risk: Impact of Exposure of Carbonated Drinks and Fruit Juice on Primary Enamel Surface and Microhardness – An *In vitro* Study

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Abstract

Aims: The purpose of this study is to evaluate and compare the microhardness and surface enamel changes on exposure to carbonated drinks and fruit juice. **Methods:** The study comprised 15 primary teeth which were cleaned with slurry of pumice and stored in deionized water. The roots were removed and the crown was cut from the distal, mesial, buccal, and lingual side. The specimens were divided into four groups according to type of beverage. Beverages used in this study are carbonated drinks (Coca Cola and Sprite) and fruit juice (Tropicana orange). Artificial saliva was used as control. All the specimens of the three groups were exposed to acid for 5 min at room temperature twice a day at 10 am and 1 pm for 5 days. After exposure, sectioned specimens were mounted and viewed under scanning electron microscope (SEM) for any surface enamel changes and were subjected to microhardness tests with Vickers diamond indenter. **Results:** Parametric test, one-way analysis of variance with *post hoc* Games-Howell test was used for statistical analysis. A significant reduction in microhardness was found in all groups exposed to carbonated beverages and fruit juice. All beverages used showed varying amounts of enamel dissolution as verified by SEM. **Conclusions:** There was a definite reduction in microhardness in all groups exposed to carbonated beverages and fruit juice. The lowest mean microhardness score was observed for orange juice and highest for sprite. Fruit juices containing citric acid were more aggressive than carbonated drinks which contain phosphoric acid.

Keywords: Beverages, Dental Erosion, Microhardness

Introduction

Prevalence of dental erosion appears to have escalated significantly and has been recognized as a crucial cause of tooth structure loss in adults and children.[1] “Dental erosion” and “erosion dentium” are the terms used to characterize the physical result of a pathologic, chronic, localized, painless loss of dental hard tissue chemically etched away from the tooth surface by acid and/or chelation without bacterial involvement.[2,3]

Tooth erosion is termed either *extrinsic, intrinsic,* or *idiopathic*. *Extrinsic erosion* is the result of exogenous acids. The most frequently consumed erosive acids are fruit acids and phosphoric acid contained in fresh fruits, fruit juices, and soft drinks.[4] More recently, ascorbic acid (Vitamin C) contained in all sorts of drinks, sports drinks, and candies has been identified as a significant cause of extrinsic erosion.[5] Gastric acid reaching the oral cavity is identified as the major cause for intrinsic erosion. This is possible when person vomits or the result of gastroesophageal reflux. *Idiopathic erosion* is the result of acids of unknown origin.[6] pH of a dietary substance alone is not predictive of its potential to cause erosion as other factors modify the erosive process. These factors are chemical (pKa values, adhesion and chelating properties, titratable acidity, calcium, phosphate, and fluoride content), behavioral (eating and drinking habits, lifestyle, and excessive consumption of acids), and biological (flow rate, buffering capacity, temperature, composition of saliva,

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pellicle formation, tooth composition, and dental and soft-tissue anatomy).[7]

Clinical studies claim that dental erosion will be more critical when teeth are exposed to acidic substance for longer time than the volume of acidic drink consumed. Moreover, the total acid level, acid type, concentration of phosphate, calcium, and fluoride in the beverages have a modifying effect on the development of erosion induced by dietary components.[8,9] Apart from chemical aspects of erosion, physical characteristics of enamel surface were evaluated to indicate the loss of tissue due to exposure to soft drinks with low pH values.

Deciduous enamel and dentin are more thinner than permanent teeth. Erosive process reaches dentin earlier in deciduous enamel when compared to permanent enamel.[10] The purpose of this study is to evaluate and compare the microhardness and surface enamel changes in primary teeth on exposure to carbonated drinks – Coca Cola and Sprite and commercially available Tetra Pack orange fruit juice.

Methods

The study comprised 15 primary teeth extracted due to pre-shedding mobility. The extracted teeth were washed thoroughly under running tap water to remove blood, saliva, and other debris. They were examined under stereomicroscope to rule out the presence of cracks. Teeth were cleaned with slurry of pumice and stored in deionized water till the experiment was started. The roots of each tooth were removed and the crown was cut (using a diamond disk under water irrigation) from the distal, mesial, buccal, and lingual side.

The specimens were divided into four groups according to type of beverage. Artificial saliva is used as control.
Group 1 – (15) Teeth which are to be exposed to acid of carbonated drink – Coca Cola
Group 2 – (15) Teeth which are to be exposed to acid of carbonated drink – Sprite
Group 3 – (15) Teeth which are to be exposed to acid of fruit juice – Orange.
Group 4 – (15) control group: Teeth which are to be exposed to artificial saliva.

The compositions and pH values of various drinks used in the study are

Coca Cola

Carbonated water, sugar, caramel, color, caffeine, phosphoric acid, and natural flavoring substances. *pH value* – 2.52.

Sprite

Carbonated water, sugar, carbonic acid, citric acid, sodium citrate, and natural flavoring substances. *pH value* – 3.29.

Tetra Pack Orange juice (Tropicana)

Water, concentrated orange juice, sugar, acidity regulator, salt, stabilizer, and natural flavoring substances. *pH value* – 3.8.

Artificial saliva was prepared in the department of biochemistry using 2 g of methyl-p-hydroxy benzoate, 10 g of sodium carboxymethylcellulose, 0.625 g of KCl, 0.059 g of $MgCl_2 6H_2O$, 0.166 g $CaCl_2 2H_2O$, 0.804 g of K_2HPO_4, and 0.326 g of KH_2PO_4 which were measured with common balance and added to 1 L of distilled water. Fluoride of 0.022 ppm was added to this solution. pH was checked with electronic digital meter and was 6.75.

The pH values of three different beverages were recorded after opening using a pH electrode. All the specimens of the three groups were exposed to acid for 5 min at room temperature twice a day at 10 am and 1 pm for 5 days. After each exposure, specimens were washed in deionized water for 20 s and immersed in artificial saliva at 37°C until the next experimental step. Artificial saliva was changed daily.

After exposure, specimens were viewed under scanning electron microscope (SEM) for any surface enamel changes and were subjected to microhardness tests with Vickers diamond indenter.

SEM study (qualitative analysis)

The morphology of surfaces was examined with a SEM (Hitachi S3700N, JAPAN). Photomicrographs of representative areas of the specimens were obtained at ×800, ×3000, ×5000, and ×10000.

Measurement of microhardness

All the specimens were mounted in cold cure acrylic with cut surface exposed. The microhardness tester standardized for Vickers hardness test was used in this study. The testing parameter of 50 g for 10 s was used. The results were tabulated and subjected to statistical analysis using Microsoft Excel software.

Results

Primary enamel has lower mineral content than does permanent enamel, resulting in faster mineral loss as carious and erosion lesions. These acidic beverages compromise the mineral content and crystalline structure of tooth surface. Since the mineral content of enamel is directly related to its microhardness, a significant reduction in microhardness was found in all groups exposed to carbonated beverages and fruit juice. In the intergroup comparison [Table 1 and Graph 1], there was overall significant difference in the mean microhardness values among the four groups. *Post hoc* test showed that Group III (249.87 ± 9.46) showed significantly lower mean microhardness score when compared to Groups I (266.93 ± 17.36), II (273.40 ± 10.70), and IV (302.47 ± 6.91) with a level of significance <0.001. Similarly, Group IV (302.47 ± 6.91) had significantly higher mean than Groups I, II, and III. Table 2 shows the intergroup comparison of the level of significance.

All beverages used showed varying amounts of enamel dissolution as verified by SEM.

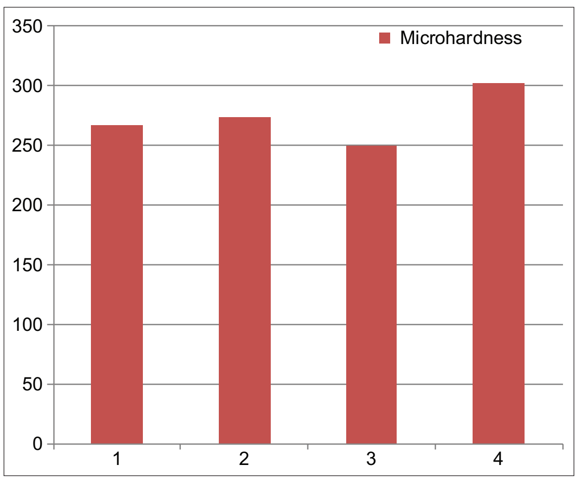
Table 1: Microhardness of teeth determined using SEM and its reduction due to different carbonated drinks

	Group 1		Group 2		Group 3		Group 4		*P*-value	*Post hoc* test
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Microhardness	266.93	17.36	273.40	10.70	249.87	9.46	302.47	6.91	<0.001; Sig	1,2>3 4>1,2,3

Sig – significant

Table 2: Intergroup comparison of level of significance

Intergroup	*P*-value
1 versus 2	0.616; NS
1 versus 3	0.015; Sig.
1 versus 4	<0.001; Sig.
2 versus 3	<0.001; Sig.
2 versus 4	<0.001; Sig.
3 versus 4	<0.001; Sig.



Graph 1: "Intergroup comparison of mean microhardness scores"

Discussion

Dental erosion according to Tencate and Imfeld (1996) "is the clinical term that is used to describe the physical results of a pathologic, chronic, localized loss of dental hard tissue that is chemically etched away from tooth surface by acid/chelation without bacterial involvement".[11] Common extrinsic factors like beverages with low pH values that cause erosion include fruit juices and fruit teas.[12]

The present study was undertaken to evaluate and compare the microhardness and primary enamel surface changes on exposure to carbonated drinks and fruit juice. We have selected two most commonly used carbonated drinks Coca Cola and Sprite and Tropicana orange (Tetra Pack fruit juice).

Cola is one of the most commonly used worldwide acidic drink (pH <4) that could significantly dissolve mineral content of enamel even in short time exposures.[13] Soft drinks caused considerable enamel demineralization below the critical pH (5.5). Viscosity, buffering capacity, and pH remain the most important chemical parameters in determining the erosive potential of soft drinks. The hydrogen ion concentration (H+) of the acidic substances which are available to interact with the tooth surface, also called the titratable acidity, is considered to be more important than the actual pH.

Fruit juices, especially orange juice, and Cola soft drinks were commonly used in similar erosion studies and were compared to the erosive potential of other beverages Gumilang *et al*.,[14] Mathew *et al*.,[15] and Enam *et al*.[16] Results for both Coca Cola and orange juice showed positive erosive response that is well supported by the literature.[17,18]

The erosive capacity in the beverages is largely affected by the ingredients present in them. Citric acid (also found in orange juice) is a crucial constituent of various acidic beverages with an erosive potential inducing enamel demineralization. Studies also cite that citric acid has higher capacity for the dissolution of the hydroxyapatite crystals because of formation of calcium citrate and loss of common ion effect due to withdrawal of Ca^{2+} from surface enamel.

Deionized water has been used in many studies as storage medium for extracted teeth. In the present study, the teeth were stored in deionized water after washing in running water, till experiment was started so that no change in the hardness of enamel is seen. Sample preparation for the present study was in accordance with Barac.[19] In the present study, we have exposed the specimens to the contents of the beverages for 5 min, twice daily for 5 days. The effects of acidic beverage consumption can be represented by demineralization treatment of 5 min.

The Vickers microhardness test was used in the present study because it has been argued that Vickers hardness number is a better indicator of the remineralization effect compared to pH value.[20] Moreover, the 50 g load was selected in the experiment as according to the available documentations, this magnitude of force would provide an appropriate size of indentation for accurate measurement.[21]

All the beverages that are used in this research are different from each other with respect to pH values, type of acid, and amount of titratable acid.

This may explain the different erosive potentials of the solutions. The least erosive loss of enamel was observed for sprite. Many studies have shown that there is a significant reduction in enamel's microhardness under acidic challenge.[22-28] In our study too, there is a significant reduction in enamel's microhardness with the exposure to carbonated drinks and fruit juice. Each of these drinks caused great changes in characteristic appearance of enamel when viewed under SEM.

Results of the present study showed that microhardness reduction was greater in Group III (orange) than Group I (Coca Cola) and Group II (Sprite). Similar findings were obtained from study conducted by Gumilang *et al*., Zimmer *et al*. (2015),[29] and Tedesko *et al*.[30] which showed that the decline in tooth enamel hardness increases with the duration of immersion in orange juice. All the drinks used in this study have acidic pH values and are potential decalcifying substances. These findings are similar with earlier reports.[31] The study conducted by Von Fraunhofer *et al*.[32] also found that beverages containing higher concentrations of citric acid have an aggressive effect on the enamel surface leading to its dissolution. The findings obtained in the present study are also in accordance with study done by Mathew *et al*.[16] and Ren *et al*.[33] which concluded that orange juice erosion markedly decreases hardness and increases roughness of enamel.

On the contrary, Rajeev *et al*.[34] evaluated the erosive effects of various beverages on enamel and cementum of deciduous and permanent teeth and showed that carbonated drinks, (which contain phosphoric acid), have higher erosive potential as compared to fruit based drinks (which contain citric acid). Similarly, Bhatia[35] studied the effect of different beverages on surface hardness of nanohybrid resin composite and found that decrease in the microhardness was more with aerated drinks than orange juice.[36,37] These varying results may be explained by the different methodologies used.

The mineral content of tooth is primarily composed of calcium-deficient carbonated hydroxyapatite. In the process of erosion by chelation and/or acids, these agents interact with the surface of the mineral crystals after diffusing through the pellicle, the plaque, and the protein/lipid coating of individual crystals. Hydrogen ion is very aggressive and has capacity to attack and dissolve the tooth minerals. This is done when H^+ ion is combined with carbonate and/or phosphate to release all the ions from crystal surface region and thus leads to direct surface etching. Acids such as citric acid have a more complex interaction.[38]

The approximate value of carbonate content in enamel is 3% while in dentine, the value is 56%. Hence, the dentine mineral is more acid soluble. Further, the crystals in dentine are much smaller than those in enamel, therefore, rendering wider surface area of dentin susceptible to acid attack. Smith and Shaw suggested that children and babies who consume fruit juice should receive vitamin supplements to prevent lesion formation on the deciduous tooth surfaces. Grenby[39] studied *in vitro* the changes on the surface of the enamel caused by eight acidic soft drinks and determined that microorganisms originating from the sugar in the drinks had an effect on demineralization of the acids and the hydroxyapatite.

In the present study, it is to be noted that specimens from different groups treated with beverages revealed some sort of different roughness patterns on examination under SEM. This could be attributed to the difference in compositions of these drinks, as reported by Lussi *et al*.[40] who studied the effect of citric and hydrochloric acids on enamel surface and found that all kinds of acids have specific erosive patterns. They further stated that citric acid is likely to give honeycomb like erosive pattern while hydrochloric acid produces characteristic loosened enamel structure. Phosphoric acid showed fish scale appearance.

In the present study, specimens in Group III (teeth immersed in orange juice) showed noticeable alteration in the tooth surface under SEM. Irregular patterns of pit-like erosion areas and honeycomb pattern of rod and inter-rod structure were seen varying from site to site. This was similar to study done by Ren *et al*. SEM showed that enamel surface exposed to Cola drinks produced an early pattern of demineralization with evidence of interprismatic mineral loss similar to study by Grewal *et al*.[41] Enamel surface of specimens in Group II (teeth immersed in Sprite) showed no specific pattern. It revealed some amount of surface irregularities and pitting.

In the present study, all the selected beverages had low pH value, with Coca Cola having the lowest at 2.52. Artificial saliva, which was used as a reference, had a pH 6.7 near to neutral/alkaline at 7.3. Greater the buffering capacity of a beverage, longer it will take for the saliva to restore the pH value. Sports and carbonated drinks have been reported to have a high buffering capacity and a low pH.[42,43]

Conclusions

- Carbonated beverages and fruit juices are markedly aggressive toward primary enamel. And showed varying amounts of enamel dissolution as verified by SEM.
- The lowest mean microhardness score was observed for orange juice and highest for sprite indicating erosive potential of orange juice was higher followed by Coca Cola and Sprite, clearly indicating that fruit juices containing citric acid were more aggressive than carbonated drinks which contain phosphoric acid and also enamel aggressivity is determined by beverage composition, type of acid rather than PH.

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