Experimental study of iron and multivitamin drops on enamel microhardness of primary tooth

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Abstract

Objectives: Iron and multivitamin drops are being frequently prescribed in children less than 2 years of age. Due to their low pH levels, these drops may lead to the softening of enamel and accelerate the destructive process. The aim of the present study was to investigate the enamel microhardness of primary teeth after exposing them to iron and multivitamin drops. Materials and Methods: Forty healthy anterior teeth were randomly divided into four groups of 10 samples each. Samples were exposed to two iron drops of Kharazmi (Iran) and Ironorm (UK) and two multivitamin drops of Shahdarou (Iran) and Eurovit (Germany) for 5 min. The surface microhardness was measured before and after exposure and data processing was done using statistical paired t-test and analysis of variance (ANOVA) test. The surface structure of the teeth was examined by scanning electron microscope (SEM). Results: In all groups, microhardness was decreased, but it was not significant in Eurovit multivitamin group (P = 0.088). The reduction rate in Kharazmi iron group was significant compared to that in other groups (P < 0.005). Hardness reduction percent for Kharazmi iron drop was 28/12 ± 47/43. In SEM analysis, irregular granular appearance was observed in the enamel exposed to Kharazmi iron drop. Conclusion: The results showed that all the studied drugs have the potential to cause erosion; this potential is the most in Kharazmi iron drop and the least in Eurovit multivitamin drops. Therefore, after using these kinds of drops, preventive measures should be used in children.

Key words: Dental erosion, iron drops, microhardness, multivitamin drops, primary teeth enamel

INTRODUCTION

A large variety of products could be suggested for the treatment of iron deficiency in children and since the absorption of Fe (II) is more effective than that of other oral iron products, its consumption is more common.¹ It is usually prescribed to prevent iron deficiency in children from 6 months to 2 years of age. Its consumption may cause black discoloration on primary tooth, and many parents think that a kind of decay has been formed after giving iron drops to their children; this may be the reason why they limit the consumption of this essential element by their children.² This dental discoloration is one of the reasons parents and children refer to dental offices.³ Demineralization of enamel has a clinical importance and many studies investigated the differences between the susceptibility of the enamel in primary and permanent teeth to erosion. In some studies [Amaechi et al. and Hunter et al.],⁴,⁵ more susceptibility to enamel erosion was observed in primary teeth (50% more mineral loss and 30% more lesion depth). The enamel of primary teeth is less mineralized than that of

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permanent teeth and the density of its outermost layer is lesser.\textsuperscript{[6]} Besides the other factors causing tooth decay, such as poor hygiene and inappropriate diet, erosion and softening of enamel can lead to the emergence of early dental caries in children. As it has been shown in the study of Kazoullis et al. in 2007, dental erosion in primary teeth is almost three times more common than in permanent teeth and it has a high relation to caries experience in children.\textsuperscript{[7]} According to some studies, drug use can cause erosion. The oldest study in this regard is by James and Parfitt conducted in 1953, in which they investigated the effects of different iron tonics on healthy teeth; after a week, they found that tooth decay was related to the acidity of the solution. The iron tonics used in this study had a pH of 1.5–8.56. This paper has recommended that iron tonics should be administered through a straw or glass tube for babies.\textsuperscript{[8]} However, in younger children, parents usually dilute the drugs and use a teaspoon to feed the baby, and the child sucks the medication on his/her upper incisors. If this continues for a long time, decalcification of upper labial incisor enamel surfaces that are often associated with dental caries will appear. If the drug also contains a sweetener, the risk of caries significantly increases. In severe cases, there is a risk of tooth fracture, and it may progress toward gums until only the root remains. But we cannot say that dental damage always occurs following the use of iron tonics. The degradation rate depends on the acidity of iron tonic, frequency, duration, method of use, but regular consumption of acidic syrups clearly has the potential to cause destruction of the tooth structure. In some studies, chewable tablets, ascorbic acid (vitamin C), and multivitamin syrup also have been found to reduce the hardness of teeth.\textsuperscript{[9]} However, some studies found that iron tonics reduced enamel demineralization. Some studies have shown that iron reduces enamel demineralization in a cariogenic condition.\textsuperscript{[10]} Therefore, we decided to investigate the effects of these pharmaceutical products on the variation in microhardness of the primary teeth. This study which was done to evaluate the effects of two types of iron drops and two multivitamin drops on the hardness of enamel in 40 primary teeth used the iron and multivitamin drops approved by the Ministry of Health that are available in drugstores.

**MATERIALS AND METHODS**

The buccal surfaces of the extracted primary teeth without caries, erosion, cracks, and enamel lesions were identified and randomly divided into four groups. First, some important chemical parameters relevant to erosion, such as titrable acidity (TA), pH, and the amount of fluoride, citric acid, calcium, phosphate, and the citrate content of the drugs, were identified at the medical biochemistry laboratories of Tehran University and Babol University of Medical Sciences. Drugs’ pH was measured by a digital pH-meter Basic 20+ (Crisom Instrument, SA, Barcelona, Spain), and Maguire method was used to determine TA.\textsuperscript{[11]} The amount of added base to attain pH 7 was measured using a pH electrode, wherein 50 ml of each drug was titrated by NaOH. 1 N calcium and phosphate were determined using a standard biochemistry kit employing the spectrophotometric method (Cecil, UK) at the biochemistry laboratory of Babol University of Medical Sciences. The amount of fluoride was analyzed using ion selective electrode and the amount of citric acid was also analyzed by UV-visible spectrophotometry (shimadrou) at the biochemistry laboratory of Tehran Medical University.

The list of examined drops was as follows:

- Kharazmi iron drop, made in Iran (containing 25 mg/ml of iron ions)
- Shahdarou multivitamin drop, made in Iran
- Eurovit multivitamin drop, Eurhovital, Germany
- Ironorm iron drop, Wallace, Britain, containing 125 mg of ferrous sulfate BP equivalent to 25 mg of iron per ml

The total number of samples was 43. Investigations were done on the labial surface of 40 extracted mandibular primary central and lateral incisors of 40 children aged between 5 and 8 years; the extraction time was not more than 3 months. Teeth were kept in a package containing saline 0.9% at room temperature until the study time. Before measuring the hardness, the teeth were embedded in polyester, so that lingual surface of the tooth crown was immersed inside a polyester mold to the extent that only the surface of crown remained out of the polyester. Then, surface preparation was performed as follows. Since the surface-intact enamel has higher amount of fluoride and higher resistance against acids than the lower layers,\textsuperscript{[12]} it was tried to polish the samples minimally by silicon carbide papers with the numbers 800, 1000, 1200, 2000, and 2500 grit under surface water pressure, until almost smooth surfaces free of scratches could be seen under a microscope. Then, the initial hardness measurement was performed using Vickers microhardness tester machine (MH1, Koopa Pazhooheesh, Tehran, Iran). At first, the labial surface of the teeth was examined and the best straight and parallel surface was determined to apply the force. Then, the indentor of hardness tester
was placed on the tooth surfaces and after imposing 50 g force for 10 s, the surface was examined again under the microscope.

A square point was created on the tooth surface and two diagonals of this square were measured by a linear which is embedded within the device (D1, D2) to obtain the average of diagonals (D); Vickers hardness number was calculated with D and P (imposed force) using the formula: \( H_v = \frac{2P}{D^2} \). Teeth with hardness in the range of 250–500 Knoop Hardness Number (KHN) equivalents to 239–478 Hardness Vickers (HV) were selected because the numbers out of this range are out of the normal range.[13] After measuring the initial hardness, 40 teeth were randomly divided into four groups of 10 teeth each and each group was immersed in the above-mentioned drops for 5 min.[14] During this period, the teeth were placed in a shaker incubator (Unimax 1010; Heidolph, Germany) at 37°C. After removal from the solution, the teeth were washed with distilled water and hardness of each tooth was measured again with a Vickers hardness tester machine. It should be noted that to obtain the exact number each time, hardness test was measured on the tooth surface at three points in same surface and the average values were considered as the hardness of samples. In order to compare the hardness of teeth, paired t-test was used before and after exposure to drops and analysis of variance was used to compare the impact of drops on the hardness of teeth; and in all samples, \( P < 0.05 \) was considered significant. At the end, 15 teeth were selected for scanning electron microscopic (SEM) study; 3 control samples without the influence of drugs, and 12 samples in four groups of 3 samples each, which were previously immersed in drops for 5 min. First, they were vacuumed and then dehydrated; then, they were covered by a very thin layer of gold (Ball-Tec, Lausanne, Switzerland) and examined by SEM (Philips, XL30; Almelo, The Netherlands) at Tabriz Modares University. It should be mentioned that this part of study was a qualitative study to assess the changes in tooth surfaces.

RESULTS

The results of laboratory analysis of some chemical parameters of drugs are as given in Table 1 and are discussed below.

The changes in pH range among the test drops varied between 3.36 in Eurovit multivitamin and 1.2 in Kharazmi iron drop. Kharazmi Iron had the highest TA (2.37) and Ironorm iron drop had the lowest TA (0.87). The amount of fluoride was much less than 1 ppm in all the drops and there was not any significant difference between them. The highest concentration of calcium was observed in Eurovit multivitamin and the highest concentration of phosphate was observed in Ironorm iron drop. The citrate rate was higher in Shahdarou than in other drugs [Table 2]. According to the electron microscopic study, the samples exposed to Kharazmi iron drop showed significantly higher porosity than other groups. In this group, some minor irregularities, porosity, cracks, and fractures could be seen on the enamel surface [Figures 1–6].

DISCUSSION

Since one of the erosion assessment methods is to measure the microhardness of enamel, the hardness of enamel in primary teeth was measured by Vickers hardness tester machine before and after exposure to test drops used in this study. As primary anterior teeth are the first teeth appear in the mouth and are important in a child’s appearance, parents realize their discoloration soon. In 2000, Lussi et al. examined the enamel microhardness of primary and permanent teeth after consumption of a number of drinks and foods, effervescent tablets, vitamin C, and multivitamin syrup; they found that all the materials under study, except for fruit yogurt, significantly reduced the hardness of enamel in primary and permanent teeth and the overall rate of reduction was 17.5 ± 27.2 KHN for primary teeth. The highest reduction was after 4 min of immersion in Sprite beverage (151.8 KHN hardness reduction in primary teeth).[15] In our study, the reduction rate was 167.34 ± 48.55 for Kharazmi iron drops, which was higher than the values obtained in the study of Lussi et al. There are some studies that examined other medicines with erosion potential, like McNally et al., in which six analgesic solutions containing citric acid were studied; they found that there was only one analgesic solution with the clinical potential for enamel erosion.[16] In 2006, Costa et al. reported on the reduction of hardness caused by antihistamine syrup in primary teeth. Consumption of this drug along with pH cycling reduced the hardness in vitro, simulating the daily dose to 46.83%. Furthermore, Sales-peres et al. studied the effect of a mouthrinse containing iron on enamel and dentin erosion. They found that there was more erosion in the enamel than in dentin and iron solution could cause significant decrease in enamel surface microhardness change (SMH %); this could be due to the precipitation of ferric
Table 1: Some chemical properties of the test drops

| Drops            | Country    | pH  | TA to pH 7 | PO4 (mmol/l) | Ca (mmol/l) | Fluoride (ppm) | Citrate (ppm) |
|------------------|------------|-----|------------|--------------|-------------|----------------|---------------|
| Eurovit multivitamin | England    | 3.36| 1.35       | 30.99        | 37.775      | 0.02           | 14            |
| Shahdarou multivitamin | Iran       | 2.3 | 1.37       | 5.79         | 10.275      | 0.03           | 498           |
| Ironorm iron     | England    | 2.61| 0.87       | 130.60       | 28.025      | 0.01           | 25            |
| Kharazmi iron    | Iran       | 2.1 | 2.37       | 119.87       | 0.375       | 0.01           | 102           |

Table 2: Teeth enamel hardness based on Vickers after exposure to iron and multivitamin drops

| Drops            | Number | Mean±SD Before | Mean±SD After | P (paired t-test) | Mean±SD (decrease) | Mean±SD (percentage of decrease) |
|------------------|--------|----------------|---------------|-------------------|--------------------|----------------------------------|
| Eurovit multivitamin | 10     | 377.44±58.22   | 48.07±346.04  | 0.088             | 51.86±31.40        | 13.97±7.15                       |
| Shahdarou multivitamin | 10     | 60.28±371.25   | 312.88±72.90  | 0.011             | 58.22±58.37        | 15.94±15.57                      |
| Ironorm iron     | 10     | 30.43±388.53   | 54.93±19.24   | 0.001             | 47.46±69.29        | 12.23±17.31                      |
| Kharazmi iron    | 10     | 39.60±386.12   | 53.67±218.78  | 0.000             | 48.55±167.34       | 12.28±43.47                      |
| P value (ANOVA test) | -      | 0.849          | 0.000         | -                 | 0.000              | 0.000                            |

Figure 1: Vickers microhardness tester machine

Figure 2: SEM images of polished enamel without the effect of drugs: (a) 500×; (b) 2000×

Figure 3: SEM image of the polished enamel which was exposed to Eurovit multivitamin drop for 5 min: (a) 1000×; (b) 2000×

Figure 4: SEM image of the polished enamel which was exposed to Shahdarou multivitamin drop for 5 min: (a) 1000×; (b) 2000×

Figure 5: SEM image of the polished enamel which was exposed to Ironorm iron drop for 5 min: (a) 1000×; (b) 2000×

Figure 6: SEM image of the polished enamel which was exposed to Kharazmi iron drop for 5 min. (a) According to this figure, some minor irregularities, porosity, cracks, and fractures can be seen on the enamel surface (1000×). (b) According to this figure, some minor irregularities, porosity, cracks, and fractures can be seen on the enamel surface (3000×)

phosphate formed by ingredient of phosphate ion that can be soluble on the enamel surface, which acts
as a barrier on the enamel and reduces tooth tissue loss.[10] In a study by Kato et al., on the effect of iron on demineralization of bovine enamel blocks after the consumption of a beverage containing 10 mmol/l iron, the wear was significantly reduced.[17] It seems that iron solution itself causes significant reduction in enamel surface microhardness changes and the loss of tooth tissue, and in fact, the low pH of drops containing iron can lead to dental erosion.

However, in the meantime, TA is more important[15] because the total amount of acid (TA) represents the actual amount of available H+ to react with the dental surfaces.[18] Acids are usually used as a buffering agent to maintain the chemical stability, for the control of consistency, or for physiological adaptations. Furthermore, acidic compounds are mostly required for the solubility of drugs. Acidic ingredients have better taste and improve patient compliance.[11,19] However, the amount of citrate was significant in Kharazmi and Shahdarou drops (498 and 102 ppm, respectively).

It seems that parameters like pH, exposure time, temperature, and type of acid could affect the strength of acidic materials to cause erosion.[20] The pH of drops used in this study was in the range of 2.1–3.3, which is below the critical pH range of enamel. Meanwhile, Kharazmi iron drop had the lowest pH and highest TA (2.37). As can be seen in the SEM photographs, this may cause the demineralization of enamel surfaces. In the SEM images of the Kharazmi iron drops, enamel erosion and porosity, cracks, and fractures were observed [Figure 5]. Although we do not know exactly what caused the pores and cracks, they may be due to the exposure to low pH or the pharmaceutical combinations or dehydration. The increase of surface roughness, however, can cause more retention of the plaque and increased susceptibility to tooth decay. Endogenous erosive potential effects of some common drugs used for children, such as salbutamol (pH = 6.05), paracetamol (pH = 6.77), and theophylline (pH = 7.71), were studied by Babu et al. using SEM; in all the samples, irregular erosion cavities were observed in teeth enamel surfaces.[21] Tupalli et al. evaluated the erosive potential of various pediatric liquid medicaments with SEM in primary teeth. All drugs used in their study (analgesics, antibiotics, anti‑epileptics, multivitamins, and anti‑tussives) showed an erosive effect on primary enamel surfaces. The majority of the medications, especially multivitamins, caused etched prism pattern followed by crater formation and sporadic rod ends. The content of calcium, phosphate, and fluoride of drugs has a protective effect.[22] In comparison to Kharazmi iron drop, Ironorm iron drop caused lower reduction in microhardness of samples due to high levels of calcium and phosphate. Among the studied drugs, the Eurovit drop was safer than others, and this can be related to the higher pH, higher amount of calcium, and lower citrate than the other drops. There was no significant difference in the amount of fluoride in the studied drops and the low levels do not seem to have a protective role. This was an in vitro study, so the role of protective factors such as saliva has not been evaluated. Perhaps in the future in vitro studies, this shortcoming can be compensated by performing pH cycling partially.[23] Eskandarian et al. studied the effect of three iron drops on the surface hardness of primary tooth in artificial decay plants; the results have shown that iron supplements have no effect on the demineralization of the teeth structure. The difference between the results of their study and the present study could be due to the different concentrations of iron drops (1 ml iron in 25 ml medium) and the difference in the procedures; for example, in their study, after immersing in solutions, the samples were polished and then microhardness was measured, which may remove the most superficial layer influenced by the drugs.[24]

Another characteristic of our study was the least polish on the enamel surfaces because our goal was to maintain the superficial outer layer of enamel with the most similarity with the oral environment. This surface was only partially smoothed to have a symmetrical indentation. Data obtained from this study can be used by physicians and dentists to provide sufficient information about the erosive power of the prescribed medications for consumers. It can be far better to use solid forms such as tablets instead of liquid form. If this is not possible, the patient should be advised to rinse the mouth immediately after taking the medicine. It is not recommended to brush the teeth right away because the enamel softened by acid treatment can be easily removed by toothbrush and toothpaste. The drug should not be taken at bedtime or between the main meals. In the case of older children, sugar‑free gum is recommended after taking these drugs.[17] According to other studies, adding xylitol, fluoride, or a combination of them to the acidic drinks or using fluoride with xylitol as a mouthwash after exposure to acidic solutions could not prevent the enamel erosion. Since acidic attacks are much stronger in erosion, the absence of mineral materials is much stronger than what happens in the process of tooth decay. This means that the mechanism of anti‑demineralization of fluoride and xylitol may not be effective enough against the erosion process.[25] Adding casein phosphopeptide...
amorphous calcium phosphate (CCP-ACP), casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF), and tricalcium phosphate fluoride (TCP-F) to beverages without affecting the taste of the drink significantly reduces the erosion. It seems that further studies are required on the protective role of these new materials. It is possible to decrease the potential for erosion in syrups by correcting the type of acid, for example, by using maleic acid instead of citric acid and by adding calcium and phosphate as supplements.

CONCLUSION

According to the result of present study, although three brands of iron products, including Kharazmi, Shahdarou and Ironorm, reduced the average enamel microhardness of primary teeth, there was a definite reduction in microhardness in the group exposed to Kharazmi drop, which was significantly different from the other groups.

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Conflicts of interest
There are no conflicts of interest.

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