

## Original Article

### Evaluation of the relationship between pH and titrable acidity of five different of iron supplements with the absorption of iron ions in the anterior primary teeth (an *in vitro* study)

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#### ABSTRACT

**Background:** Iron supplement is recommended for alleviating iron deficiency. Black discoloration of teeth is one of the side effects of consumption of iron supplements. Besides these effects, iron drops may also act directly on the dental hard tissues, with erosive lesions as possible outcomes of their content. In this regard, we decided to compare iron absorption of five types of iron drops in enamel and determine its relationship with pH and titrable acidity (TA) of them.

**Materials and Methods:** In this *in-vitro* study The pH of 5 kinds of iron was measured by a digital pH meter. Each drop was titrated and then the TA was calculated. For this experimental study, 40 healthy anterior primary teeth were prepared and divided into 5 groups then exposed to iron drops. The level of iron absorption was determined by atomic absorption. The Pearson correlation test was used to analysis correlations between pH, TA, and absorption of iron ions in the anterior primary teeth. One-way ANOVA was used for statistical analysis. Statistical significance was set at  $P < 0.05$ .

**Results:** The mean of pH in Kharazmi drops was significantly less than both Irovit, and FerroKids drops ( $P < 0.05$ ) but did not differ significantly with other drops. Kharazmi drops TA was higher than all drops ( $P < 0.05$ ). There was no significant difference between the mean iron ion adsorption, as well as between iron ion absorption with pH and TA also not found a statistically significant relationship.

**Conclusion:** All drops have acidic content that increases their potential for erosion. Reducing the potential of the effects of iron drops on dental hard tissues should be of concern to all health professionals.

**Key Words:** Acidity, deciduous tooth, iron

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## INTRODUCTION

Iron deficiency anemia is a major health issue that occurs worldwide in both developed and developing countries. Approximately 50% of anemia cases are

caused by iron deficiency.<sup>[1]</sup> The overall prevalence of the anemia in Iranian children with under 5 years old was estimated about 18%–38%.<sup>[2]</sup>

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Iron plays an important role in the development of nervous system, especially during growth years. Iron deficiency anemia has adverse effects on various health conditions such as physical, behavioral, and cognitive disorders in preschool and school children.<sup>[3]</sup> In early childhood, anemia can be due to bad feeding habits, especially during breast milk is replaced by foods that are poor in iron and other nutrients, including Vitamin B12 and folic acid.<sup>[4]</sup> In all countries, the program of iron supplementation is carried out for free for the low-income people. In Iran, as a national health policy to prevent iron deficiency anemia, the supplementary programs have been provided for 6–24-month-old babies and include administration of daily 15 drops of iron. Despite the importance and high costs of this act, inadequate or irregular intake of iron supplementation has been widely reported.

Various reasons have been suggested for this issue among which, black discoloration or undesirable changes in deciduous teeth as the result of iron supplement is the most important reasons in avoiding iron supplement usage for children by parents.<sup>[5]</sup>

The potential effect of iron ion on development and progression of dental caries, enamel decalcification, concentration of saliva, stains, and oral microbial flora are evaluated in previous human and animal studies.

Liquid dosage form of iron supplement usually prescribed for children to aid compliance. To maintain chemical stability, control tonicity, physiological compatibility in pharmaceutical preparations and to improve flavor, acids are frequently used as buffering agents to liquid oral medicines.<sup>[6,7]</sup> Acids in medicines may also act directly on dental hard tissues and increase their solubility.<sup>[8,9]</sup>

Pasdar evaluated the pH and titrable acidity (TA) of liquid oral medicines for children and found ferrous sulfate drop has the acid content and may help to prolong the pH drop after consumption. This medicines may result in erosion of teeth.<sup>[10]</sup>

Shabzendehtar *et al.* compare of primary enamel discoloration caused by the use of three different iron drops and noticed the more increased surface by etching in teeth effects the more change in color and this was most prominent in Iranian iron drop.<sup>[11]</sup>

In the study of Martinhon *et al.* that evaluated the effect of ferrous sulfate on the reduction of demineralization of blocks of enamel and changing

the ionic composition of the formed biofilm. He demonstrated that in a cariogenic environment, ferrous sulfate significantly decreased enamel demineralization, and the percentage of surface microhardness change in enamel blocks.<sup>[12]</sup>

Therefore, reporting positive, negative, and sometimes controversial results of iron on teeth in the previous study, we decided to investigate the endogenous pH and titratable acidity of five types of iron supplements and evaluate the relationship of them with the absorption of iron ions in the anterior primary teeth.

## MATERIALS AND METHODS

In this *in-vitro* study, first TA and pH of the drugs were identified at the medical biochemistry laboratories of Ardabil University. Drugs' pH was measured by a digital pH-meter, and Maguire method was used to determine TA.<sup>[13]</sup> A volume of 2 ml of drugs dissolved in 40 ml distilled water and the amount of added base (NaOH 0.1 M) to attain pH 7 was measured using a pH electrode. PH measurement and titration were repeated three times for each iron supplement.

The list of examined drops was as follows

1. Ferrous sulfate (Kharazmi, Iran) containing 25 mg/ml of iron ions
2. Ferbolin (Shahdarou, Iran) containing 25 mg/ml of iron ions
3. Feriron iron drop (Daroupakhsh, Iran) containing 25 mg/ml of iron ions
4. Irovit iron drop, (Vitane Pharmaceuticals Inc, Germany) containing 15 mg/ml of iron ions)
5. FerroKids iron drop, (Eur OTC Pharma, Germany) containing 16 mg/ml of iron ions.

The buccal surfaces of the total of 40 anterior deciduous teeth without caries, erosion, cracks, and enamel lesions were identified and randomly divided into five groups. All collected teeth extracted because of space discrepancy, mobility, or trauma. Teeth were stored in distilled water at room temperature until the conduction of experiment. For the preparation of specimens, the teeth were dissected at the cemento-enamel junction and the pulpal residues in the pulp chamber were completely removed. Pulp chamber was then filled with composite resin. A label was placed on the buccal surface of prepared teeth measuring 0.4 cm × 0.4 cm. The entire area surrounding the label was coated with nail polish. The label was then removed, and the glue residues were washed off with a gauze and water.

The specimens of each group were immersed in 1 mL of iron drop for 3 min and then transported to 1 mL of iron drop and 1.5 ml distilled water. The samples washed with 10 ml distilled water and immersed in hydrochloric acid 2M for 24 h. During this period, samples are occasionally shaken. After the removal of teeth from the solution, the level of iron absorption in etch group and the intact one was determined by Atomic absorption in 248.3 nanometers long wave and resolution of 0.062 ppm.

Kolmogorov–Smirnov was applied to test normal distribution of the data. The mean values were analyzed the 5 understudy groups using one-way ANOVA. The level of significance was 5%.

The *post hoc* Tukey test used to show differences between groups. The Pearson correlation test was used to analysis correlations between pH, TA and absorption of iron ions in the anterior primary teeth.

## RESULTS

According to statistical test, the distribution of collected date was normal ( $P > 0.05$ ). All of five iron drop were analyzed for pH and TA. The pH values ranged from 1.88 (Kharazmi iron drop) to 2.35 (FerroKids iron drop) [Table 1]. The all evaluated drops have acidic medium in their composition, which results in a low pH and the potential of enamel erosion increases. It was found that the mean values of pH in Kharazmi iron drop were significantly lower than in comparison with Irovit and FerroKids iron drops ( $P = 0.013$  and  $0.014$ , respectively) but it was not significantly different between other iron drops.

The results of TA were shown in Table 2. Kharazmi iron drop exhibited the largest TA compare with other drugs followed by FerroKids ( $P < 0.05$ ). Irovit iron drop had lowest TA. Shahrdaro and Daroupakhsh had similar TA ( $P = 0.628$ ).

The results of iron absorption were shown in Table 3. No significant difference was found when the five groups were compared in the level of iron absorption, but it was higher in Daroupakhsh iron drop. Between iron ion absorption with pH and TA also not found a statistically significant relationship.

## DISCUSSION

The objective of this study was to investigate pH and TA of different iron supplement products on the

**Table 1: Mean and standard deviation of pH in each test group**

Test groups	Mean	SD	P
Daroupakhsh	2.0483	0.08110	0.005
Kharazmi	1.8829	0.10372	
Irovit	2.3783	0.33944	
Shahdarou	2.0617	0.04579	
FerroKids	2.3543	0.41999	

SD: Standard deviation

**Table 2: Mean and standard deviation of titrable acidity in each test group**

Test groups	Mean	SD	P
Daroupakhsh	1.1220	0.04604	<0.001
Kharazmi	1.560	0.04604	
Irovit	0.9833	0.09973	
Shahdarou	1.1840	0.05225	
FerroKids	1.3600	0.07950	

SD: Standard deviation

**Table 3: Mean and standard deviation of iron absorption in each test group**

Test groups	Mean	SD	P
Daroupakhsh	24.9475	6.59590	0.383
Kharazmi	22.1025	5.35791	
Irovit	18.9800	6.01774	
Shahdarou	23.4138	12.0095	
Ferrokids	18.9625	3.52363	

SD: Standard deviation

effect of iron absorption to the primary anterior teeth. We observed the pH of all drugs were lower than of critical pH for demineralization of enamel and lowest pH of 1.882 was found in Kharazmi iron drop.

TA of drugs is more important factor in determination of the degree of tooth damage because that represents the total amount of available H<sup>+</sup> to react with the dental surfaces.<sup>[13]</sup> Our results confirmed the results of the previous studies and agreed with the evaluation of pH and titratable acidity of iron and multivitamin drops. They found this drugs have low pH and can reduced the average enamel microhardness of primary teeth.<sup>[14]</sup>

Several investigations have previously offered the inhibitory effect of iron on demineralization of teeth if it used iron varnish or gel form. It was concluded that iron may have cariostatic effect on the *in vitro* development of dental caries in human teeth.<sup>[15,16]</sup> Sales-Peres evaluated the effect of a mouth rinse containing iron on enamel and dentin erosion. They observe that the enamel presented more wear than

dentine and rinsing with an iron solution could cause significant decrease in enamel surface microhardness change; this could be due to the precipitation of ferric 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.<sup>[17]</sup>

Mehran *et al.* immersed teeth into the artificial caries challenge for 2 weeks before getting exposure to iron drops and observe that caused more iron absorption, severe discoloration, and structural changes in the enamel of primary teeth. These changes were more distinct in the teeth exposed to Kharazmi iron drop than others.<sup>[18]</sup> However, we did not found relationship between iron ion absorption with pH and TA of drugs, but Kharazmi iron drop has low endogenous pH that can explain the increased effect of artificial caries challenge in Mehran *et al.* study.

Eskandarian *et al.* studied the effect of different iron drops on the surface hardness of primary tooth in artificial caries environment and found that iron supplements have no effect on the change of the teeth microhardness.<sup>[19]</sup>

Peres used 10 mmol/lit concentration of ferrous sulfate in mouth rinse form and observed that exposure to ferrous sulfate after the development of erosion can reduce the dissolution of enamel and dentin structure.<sup>[17]</sup>

Results from our study confirmed the endogenous pH values varied between 1.882 and 2.378 and may have the potential to cause erosion. Considering the result of the study it seems that iron itself causes significant reduction in demineralization of enamel surface but the low pH of drops containing iron can lead to dental erosion.

Black discoloration of deciduous teeth due to iron supplement is the most important reasons of parents in avoiding of this supplement in their children's diet. Several investigations have confirmed issue.<sup>[18,20,21]</sup> Shabzندهدار *et al.* found that the absorption of iron in etched teeth increased which might have been due to increased surface therefor the more change in color was observed and this was most prominent in Iranian iron drop.<sup>[11]</sup> In this study, the level of iron absorption in Daroupakhsh and Irovit was higher and lower respectively, but no statistically significant difference was detected between iron absorption among drugs. We do not find a correlation between iron absorption and pH of drops. The difference between the results of Shabzندهدار and the present study could be due to they use from phosphoric acid for etching.

In this study, supplements were used in prepared therapeutic dosages. Kharazmi, Daroupakhsh Shahdarou iron drop in each ml contained 25 mg iron ion. Irovit in each ml contained 15 mg iron ion. The difference in dosages of iron in drops could be explain the different iron absorption. However, Irovit and Ferrokids have low iron absorption, but they cannot supply enough iron for babies in our country.

## CONCLUSION

According to the result of the present study, pH of all iron drops was lower than critical pH for enamel, and they have the potential for erosion. A public health policy must be implemented to change in composition of medicines example adding calcium and phosphate as supplements or casein phosphopeptide-amorphous calcium phosphate to reducing the potential for erosion of iron in drops. The iron absorption rate of Iranian drops is more than foreign drops; this is due to the higher concentration of iron ions in Iranian drops.

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## Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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