ARTIGO ORIGINAL

AVALIAÇÃO DO EFEITO DO SULFATO FERROSO NA DESMINERALIZAÇÃO DO ESMALTE DE DENTES DECÍDUOS HUMANOS: ESTUDO IN VITRO

EVALUATION OF THE EFFECT OF FERROUS SULFATE ON ENAMEL DEMINERALIZATION OF HUMAN DECIDUOUS TEETH: AN IN VITRO STUDY

Johnny Holanda de Gauw[1], Lara Maria Melo Costa[2], Rodrigo Neves Silva[3], Natanael Barbosa Santos[4], Maria Dânia Holanda Tenorio[5]

[1]MSc in Orthodontics. Professor at the Tiradentes University Center. Maceió, Alagoas, Brazil. johnnydegauw@hotmail.com
[2]Dentist at the Municipality of Capela, Brazilian Family Health Program. Capela, Alagoas, Brazil. larammc@hotmail.com
[3]PhD in Stomatopathology. Postdoctoral internship at the State University of São Paulo. Ribeirão Preto, São Paulo, Brazil. rodrigoneves.rn@hotmail.com
[4]PhD in Odontology. Professor at the Federal University of Alagoas. Maceió, Alagoas, Brazil. nbs.odonto@hotmail.com
[5]PhD in Odontopediatrics. Federal University of Alagoas. Maceió, Alagoas, Brazil. daniaholanda@hotmail.com

RESUMO | Objetivo: Este estudo objetivou avaliar o efeito do sulfato ferroso (SF) em dentes decíduos humanos desmineralizados e não desmineralizados. Além disso, avaliou-se a extensão da penetração do SF e seu efeito remineralizante no esmalte de dentes decíduos usando microscopia de luz polarizada (MLP). **Método:** A amostra foi formada por 44 dentes decíduos humanos. As 44 coroas foram divididas aleatoriamente em quatro grupos: A (SF após desmineralização), B (SF sem desmineralização), C (apenas desmineralização) e D (grupo controle). O SF a 0,45 mol/L^{-1} foi utilizado diariamente (15 dias) e a desmineralização foi feita por ciclagem de pH (7 dias). Em seguida, três fatias longitudinais das coroas foram fotografadas usando MLP. O grau de penetração da lesão ou mancha foi medido em micrômetros, bem como a distância entre a superfície externa do esmalte e o núcleo da lesão. **Resultados:** O grupo A mostrou uma mancha escurecida na superfície externa do esmalte maior do que o grupo B. Sugere-se, um efeito remineralizante na comparação dos grupos, A e C. A profundidade média e o desvio padrão para os grupos A, B e C foram de 4,27 µm (± 1,49), 3,72 µm (± 1,68) e 5,00 µm (± 1,84), respectivamente. Não foram observadas manchas escurecidas no grupo D. **Conclusão:** O SF manchou os dentes decíduos humanos desmineralizados e não desmineralizados. No entanto, manchas escurecidas nos dentes não desmineralizados foram menores ou ausentes, do que nos dentes desmineralizados. Portanto, o SF pode ter um efeito protetor contra a desmineralização.

Palavras-chave: Sulfato ferroso, deficiência de ferro, cárie dentária, dentes decíduos.

ABSTRACT | Objective: This study aimed to evaluate the effect of ferrous sulfate (FS) on demineralized and non-demineralized human deciduous teeth. Additionally, it was evaluated the penetration extent of FS and its remineralizing effect on the enamel of deciduous teeth using Polarized Light Microscopy (PLM). **Method:** The sample comprised 44 human deciduous teeth. The 44 crowns were divided randomly into four groups: group A (FS after demineralization), group B (FS without demineralization), group C (only demineralization), and group D (control group). FS at 0.45 mol/L^{-1} was used daily (15 days) and demineralization was done by pH cycling (7 days). Then, three longitudinal slices of the crowns were photographed using PLM. The degree of penetration of the lesion or stain was measured in micrometers, as well as the distance between the external enamel surface and the core of lesion. **Results:** Group A showed a dark stain on the outer surface of enamel larger than the group B. It is suggested, a remineralizing effect when comparing groups, A and C. The mean depth and standard deviation for groups A, B, and C were 4.27 µm (±1.49), 3.72 µm (±1.68) and 5.00 µm (±1.84), respectively. No dark stains were observed in group D. **Conclusion:** FS stained the demineralized and non-demineralized human deciduous teeth. However, dark stains in the non-demineralized teeth were smaller or absent, than in the demineralized teeth. Therefore, FS may have a protective effect against demineralization.

Keywords: Ferrous sulfate, iron deficiency, dental caries, deciduous teeth.

Submitted 20/20/2017, accepted for publication 2017/09/2017 and published 09/29/2017
DOI: 10.17267/2238-2720revbahianaodonto.v8i3.1549

INTRODUCTION

Iron-deficiency anemia (IDA) is one of the main public health issues in developing countries, such as South America, and it affects almost 2 billion people around the world, especially, preschool children[1].

In Brazil, IDA is a major health problem, and its occurrence varies between 40% and 60% among children less than 2 years old[2-4]; among children with anemia aged between 12 and 16 months, 95% have iron deficiency[2].

Prevention and treatment programs for IDA recommend the use of ferrous sulfate (FS) as an oral nutritional supplement. Because it is a low-cost treatment, has high absorption and solubility, and improves the nutritional status quickly, this preventive treatment starts at an early age. Iron supplements are well tolerated by most infants, have no measurable effect on body growth in children, and early iron supplementation, even in breastfed infants, is feasible and transiently increases iron status[4].

FS has been analyzed in several studies in dentistry[5-13] using artificial dental models (mainly bovine teeth and human permanent teeth). The most frequently reported properties include reduction in the degree of enamel dissolution[5,8,13], reduction of dental erosion[7,12], alterations in biofilm composition (in situ)[9,10,14], and oxidative modifications in glucosyltransferase promotion[11]. Another study focused on the cariostatic effect of FS prescribed in vitamin supplements containing iron[6].

Despite the benefits of FS to human health, some studies have mentioned the occurrence of dark stains, mainly in the crown of deciduous teeth, as an important side effect of FS administration[6,15-17].

In some cases, the stains prevent the intake of FS; some parents do not give FS to children with IDA because they not want their children to have dark teeth; and other parents have the misconception that iron supplements cause dental caries in children[15, 18].

Due to social disparity, there is a high frequency of IDA in Brazilian children[2,3], and pediatricians commonly prescribe FS supplementation[6,18]. The present study aimed to evaluate the effect of FS on demineralized and non-demineralized human deciduous teeth. The research hypothesis was that the presence of staining on the enamel surface by iron supplements has a protective effect against demineralization.

MATERIAL AND METHODS

This study was approved by local ethical committee for research of Federal University of Alagoas (protocol number 017311/2010-39) and all teeth scheduled for extraction were used after the patients gave informed consent. Deciduous upper incisor and/or canine teeth were donated by the Human Teeth Bank of the Dental School of Federal University of Alagoas, Brazil.

SELECTION AND TEETH PREPARATION

Evaluation of the presence of any cracks or defects in the crown structure and tooth color was previously performed using a stereomicroscope (EMZ-TR, Meiji Techno, Saitama, Japan). Forty-four homogeneous teeth were selected, cleaned and stored using thymol at room temperature until use. The crowns were separated from the roots at the cementum edge using a diamond disk (Extec, Enfield, CT, USA) attached to a model 650 metallographic cutting machine (Southbay Technology, San Clemente, CA, USA). The pulp chambers were sealed with composite resin (Fill Magic A2, Vigodent, Rio de Janeiro, Brazil). Adhesive tape was placed over the buccal side of the middle third of the crowns and the entire crown surface was painted with red nail varnish. Thereafter, the tape was removed leaving an exposed area of enamel (4×4 mm). On the lingual side, an 0.8-mm orthodontic wire was fixed in place with composite resin to allow submersion of the crowns in the test substances.

DISTRIBUTION IN GROUPS AND EXPERIMENTAL CARIES MODEL

The 44 crowns were randomly assigned into four groups, two experimental (A and B) and two control groups (C and D), each group with 11 specimens (Table 1). After receiving prophylaxis with pumice and water, the samples were submitted to the following: groups A and C were submitted to pH cycling for 7 days, following previous applied methods[19,20]. During the initial 5 days, the crowns were maintained in a demineralizing solution (2.0 mM calcium, 2.0 mM phosphate in 0.075 M acetate buffer, pH 4.3) at 37°C for 3 h (60 ml per block), and in a remineralizing solution (1.5 mM calcium, 0.9 mM phosphate, 150 mM of KCl in 0.1 M Tris buffer, pH 7.0) for 21 h (30 ml per block). On the last two cycling days, the groups were submitted only to the remineralizing solution for 48 h. When the solutions were changed, the crowns were washed with distilled water and dried on paper towels. The solutions were changed daily to prevent exhaustion or saturation and accumulation of enamel dissolution subproducts.

SUBMISSION OF SAMPLES TO TEST SUBSTANCES

After pH cycling, groups A and B were treated with the FS (0.45 mol/L^{-1} FeSO4) prepared daily in the laboratory for 15 days. The crowns were immersed in 1 ml of this solution using Eppendorf® tubes and placed in the agitation incubator (TE-420, Tecnal, Piracicaba, São Paulo, Brazil) with rotational movements at 100 rpm, 37°C, for 10 min, following previous applied methods[21]. They were then washed with distilled water, dried on paper towels, and kept in artificial saliva (1.5 mM Ca, 3.0 mM P, 20.0 mM $NaHCO^3$, pH 7.0), 1.0 ml per block.

The experiment was repeated daily until the end of the 15th day. The same experiment was performed on group D, but distilled water was used instead of FS.

PREPARATION OF HISTOLOGICAL SLIDES

On the 16th day, the crowns were cut longitudinally into three sections using a diamond disk (Extec, Enfield, CT, USA) attached to a model 650 metallographic cutting machine (Southbay Technology, San Clemente, CA, USA). Each section was trimmed using grain sandpaper (400 and 600) to approximately 150 µm. They were mounted on glass slides and kept in distilled water for 24 h.

Table 1. Distribution of deciduous teeth crowns in the control and experimental groups (n=44)

Group	FS*	Demineralization†	Teeth
A	+	+	11
B	+	–	11
C	–	+	11
D	–	–	11

+, Presence of FS; –, absence of FS or the use of distilled water.
*Solution of FS at 0.45 mol/L^{-1} daily for 15 days.
†Demineralization was done by pH cycling for 7 days.

ANALYSIS BY POLARIZED LIGHT MICROSCOPY

A microscope (BA 300, Motic, Richmond, Canada) with a polarizing filter (SW0199UH) and a 10× lens was used to view the sections. The images were analyzed using specific software (Bel Image Analyser Software, version 2.3 for Windows®). The depth of the lesions was measured in micrometers (µm) by the distance between the external enamel surface and the core of lesion.

The measurements were made in triplicate and the average was obtained. One investigator examined the central areas of each section and photographed then. Descriptive analysis of the numerical, nominal

and categorical variables was performed (absolute and percentage distributions; central tendency; dispersion).

STATISTICAL ANALYSIS

For the inferential analysis, the Kolmogorov–Smirnov test was used to determine normality and a nonparametric test was used to compare the results between the groups (Kruskal–Wallis test). The level of significance was 5% ($p<0.05$). The Statistical Package for Social Science (SPSS 14.0; SPSS Inc, Chicago, IL, USA) was used to do the calculations.

RESULTS

ANALYSIS BY POLARIZED LIGHT MICROSCOPY

The microscopic analysis of the samples of group A (submitted to FS and demineralized) revealed a dark stain on the outer enamel surface. Also, it was observed below this dark stain in group A, a mineralized region by the blue and green filters (Fig. 1a). In samples of group B (submitted to FS and non-demineralized), a dark stain was observed whether a polarizing filter was used or not, which characterizes the penetration of FS into the tooth structure (Fig. 1b). In the samples of group C (non-submitted to FS and demineralized), there was a dark stain associated with demineralization caused by pH cycling (Fig. 1c). In group D (non-submitted to FS and non-demineralized), only the dental structure with normal regions of mineralized enamel was observed (Fig. 1d).

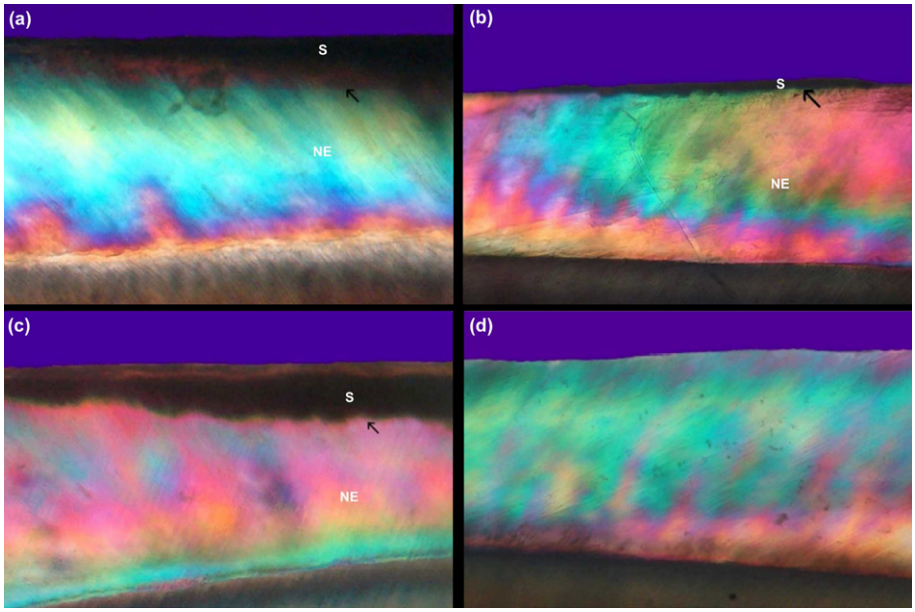

Fig. 1. (a) Photomicrograph of a longitudinal section of enamel from demineralized human deciduous teeth (group A) viewed using polarized light microscopy (10×), showing a dark stain caused by experimental caries and FS. The boundary between the demineralized area and the stain is shown (dark arrow). (b) Photomicrograph of a longitudinal section of enamel from non-demineralized human deciduous teeth (group B) viewed using polarized light microscopy (10×), showing the dark stain as a result of the penetration of FS. The boundary between the stain and the normal enamel structure is shown (dark arrow). (c) Photomicrograph of a longitudinal section of enamel from demineralized human deciduous teeth (group C) viewed using polarized light microscopy (10×), showing the dark stain. The boundary between the demineralized area and the normal enamel structure is shown (dark arrow). (d) Photomicrograph of a longitudinal section of enamel from sound human deciduous teeth (group D) viewed using polarized light microscopy (10×), showing non-demineralized areas. S: stain by FS. NE: normal enamel.

PENETRATION EXTENSION OF FS

The mean depth and standard deviation for groups A, B and C were 4.27 µm (±1.49), 3.72 µm (±1.68) and 5.00 µm (±1.84), respectively. No dark stains were observed in group D. The depth of the dark stain (the distance between the external enamel surface and the core of the lesion), identified by polarized light microscopy and recorded for each group, is showed in Figure 2.

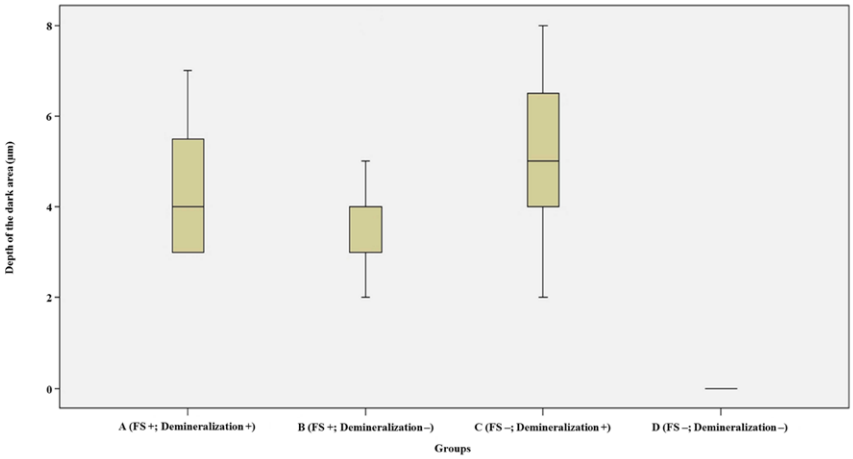

Fig. 2. Distribution of the depth of the dark stain (µm) in the groups (n=44) using polarized light microscopy. No dark stains were observed in group D. The (+) symbol indicates the presence and (–) indicates the absence of FS. A solution of FS at 0.45 mol/L^{-1} was used daily for 15 days and demineralization was done by pH cycling for 7 days (Kruskal–Wallis test, p=0.173).

REMINERALIZING EFFECT OF FS ON TOOTH DECAY ALONE AND AFTER DEMINERALIZATION

In the presence of FS, it was observed that the dark stain was bigger in group A (mean = 4.27 µm) than in group B (mean = 3.72 µm), although there was no statistically significant difference among the depth averages of these dark stains (p = 0.338).

Comparing groups, A and C, the presence of FS (group A) suggested a remineralizing effect, because the dark stain was smaller in group A than in group C, although there was no statistically significant difference (p = 0.284).

DISCUSSION

Dark stained teeth are cited in the literature as a collateral effect of FS use[16,22,23]. However, there is limited information about the effect of FS on deciduous teeth. In the current study, dark stains were seen in groups A and B, which were treated with FS. The teeth in groups A and B were demineralized and non-demineralized, respectively. Additionally, considering that a demineralized tissue becomes more permeable and increases the ion diffusion process, as it occurs in dental caries, it was possible to observe that the penetration of FS in the concentration of 0.45 mol/L^{-1} occurred more easily in the samples submitted to the cariogenic challenge, presenting an inhibitory effect on the demineralization of deciduous enamel. This was observed microscopically by the greater depth of the dark stain (group A = 4.27 µm) than the group that did not undergo to pH cycling (group B = 3.72 µm), although there was no statistically significant difference between these means (p> 0.05).

Microscopic analysis of the teeth structures showed that absorbed substances (FS or elementary iron) may be present in the enamel, and could spread beyond the enamel limits, leading to deeper dark stains. The stains occur due to iron oxidation, which occurs during its exposure to the oral environment, leading to a brownish enamel surface[17].

Group A showed a larger dark stain. Comparing the demineralized areas in groups A and C, although this area was greater in group C than in group A, there was no statistically significant difference between them ($p > 0.05$). Thus, the FS present in group A could have led to an enamel remineralization effect. This effect may be due to the formation of ferric phosphate as a result of the reaction between elementary iron and phosphate dissolved from the enamel[13]. Thus, FS may have two effects when ingested: combating IDA and inactivating early carious lesions in anemic children[24]. Thereby, the literature has shown that after the acid challenge and in the presence of FS, the ferric and phosphates ions dissolved in the surface of the enamel are precipitated and an acid-resistant surface is established in the enamel. It is possible that in anemic children treated with FS and presenting caries, these lesions may become inactive[7-9,13,24].

Many studies have shown the benefits of FS on tooth enamel[5,6,9]. A previous work subjected bovine enamel blocks to cariogenic challenges and to FS (15 $mmol/L^{-1}$) and they reported a significant reduction in mineral loss of enamel and a change in phosphate concentration at the biofilm[9], whereas another work evaluated the cariostatic effect of iron supplements at different concentrations (100% and 50%) on extracted human permanent teeth and observed a reduction in enamel demineralization after their use[6]. Moreover, other authors suggest that FS at 18 µg/mL compared with different concentrations (0.33, 0.840, and 70.0 µg Fe/mL) had better results in reducing enamel demineralization in bovine enamel[5].

As a possible clinical implication, we observed that the staining of deciduous teeth (brownish stains) appeared within 15 days after exposure to FS. However, this nutritional supplement is commonly prescribed for children between 3 months and 2 years of age[25]. Further studies may elucidate which dental products can minimize or remove these stains.

The current study sought to evaluate the effect of FS on deciduous teeth. The results show that FS, which is a commonly prescribed nutritional supplement in Brazilian public health centers as a preventive and curative therapy for children with IDA, can stain demineralized and non-demineralized deciduous teeth, however, the difference between them was not statistically significant. The relevance of this study is the analysis of the topical side effect of FS, which is prescribed for children with iron deficiency anemia, as preventive and curative therapy.

CONCLUSION

An interesting find to be highlighted in this study is the penetration of the FS into the enamel in the absence of the dental biofilm, suggesting the combination of the iron with the enamel ions. This fact may be related to intrinsic stains in deciduous teeth. In conclusion, FS can stain demineralized and non-demineralized deciduous teeth. However, the dark stained areas were smaller or absent in non-demineralized teeth, than in demineralized teeth. Additionally, when groups A and C were compared, the presence of FS (group A) suggested a remineralizing effect, because the dark stain was smaller in group A, than in group C. Therefore, FS may have a protective effect against demineralization.

AUTHORS CONTRIBUTION

de Gauw JH performed the experiments, designed the research study, analysed data and wrote the manuscript. Costa LMM performed the experiments, designed the research study, analysed data and wrote the manuscript. Neves-Silva R analysed data and wrote the manuscript. Santos NB designed the research study, analysed data. Tenorio MDH designed the research study, analysed data, wrote and correct the manuscript.

COMPETING INTERESTS

No financial, legal or political competing interests with third parties (government, commercial, private foundation, etc.) were disclosed for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.).

REFERENCES

1. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr. 2009;12(4):444-54. doi: 10.1017/S1368980008002401

2. Bortolini GA, Vitolo MR. Relationship between iron deficiency and anemia in children younger than 4 years. J Pediatr. 2010;86(6):488-92. doi: 10.2223/JPED.2039

3. Silla LM, Zelmanowicz A, Mito I, Michalowski M, Hellwing T, Shilling MA, et al. High prevalence of anemia in children and adult women in an urban population in southern Brazil. PLoS One. 2013;8(7):e68805. doi: 10.1371/journal.pone.0068805

4. Lima AC, Lima MC, Guerra MQ, Romani SA, Eickmann SH, Lira PI. Impact of weekly treatment with ferrous sulfate on hemoglobin level, morbidity and nutritional status of anemic infants. J Pediatr. 2006;82(6):452-7. doi: 10.2223/JPED.1568

5. Alves KM, Franco KS, Sassaki KT, Buzalaf MA, Delbem AC. Effect of iron on enamel demineralization and remineralization in vitro. Arch Oral Biol. 2011;56(11):1192-8. doi: 10.1016/j.archoralbio.2011.04.011

6. Al-Shalan TA. In vitro cariostatic effects of various iron supplements on the initiation of dental caries. The Saudi dental journal. 2009;21(3):117-22. doi: 10.1016/j.sdentj.2009.05.001

7. Sales-Peres SH, Pessan JP, Buzalaf MA. Effect of an iron mouthrinse on enamel and dentine erosion subjected or not to abrasion: an in situ/ex vivo study. Arch Oral Biol. 2007;52(2):128-32. doi: 10.1016/j.archoralbio.2006.08.010

8. Buzalaf MA, de Moraes Italiani F, Kato MT, Martinhon CC, Magalhaes AC. Effect of iron on inhibition of acid demineralisation of bovine dental enamel in vitro. Arch Oral Biol. 2006;51(10):844-8. doi: 10.1016/j.archoralbio.2006.04.007

9. Martinhon CC, Italiani FM, Padilha PM, Bijella MF, Delbem AC, Buzalaf MA. Effect of iron on bovine enamel and on the composition of the dental biofilm formed "in situ". Arch Oral Biol. 2006;51(6):471-5. doi: 10.1016/j.archoralbio.2005.10.003

10. Pecharki GD, Cury JA, Paes Leme AF, Tabchoury CP, Del Bel Cury AA, Rosalen PL, et al. Effect of sucrose containing iron (II) on dental biofilm and enamel demineralization in situ. Caries Res. 2005;39(2):123-9. doi: 10.1159/000083157

11. Devulapalle KS, Mooser G. Glucosyltransferase inactivation reduces dental caries. J Dent Res. 2001;80(2):466-9. doi: 10.1177/00220345010800021301

12. Yip R, Dallman PR. The roles of inflammation and iron deficiency as causes of anemia. Am J Clin Nutr. 1988;48(5):1295-300.

13. Torell P. Iron and dental caries. Swed Dent J. 1988;12(3):113-24

14. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev. 1986;50(4):353-80.

15. Talebi M, Parisay I, Mokhtari N. The parents' knowledge and behavior towards the effects of using iron supplements on tooth staining and dental caries in Mashhad, Iran. Dent Res J (Isfahan). 2012;9(6):715-8.

16. Gasparetto A, Conrado CA, Maciel SM, Miyamoto EY, Chicarelli M, Zanata RL. Prevalence of black tooth stains and dental caries in Brazilian schoolchildren. Braz Dent J. 2003;14(3):157-61. doi: 10.1590/S0103-64402003000300003

17. Reid JS, Beeley JA, MacDonald DG. Investigations into black extrinsic tooth stain. J Dent Res. 1977;56(8):895-9. doi: 10.1177/00220345770560081001

18. Eshghi A, Kowsari-Isfahan R, Rezaiefar M, Razavi M, Zeighami S. Effect of iron containing supplements on rats' dental caries progression. J Dent (Tehran). 2012;9(1):14-9.

19. Vieira AE, Delbem AC, Sassaki KT, Rodrigues E, Cury JA, Cunha RF. Fluoride dose response in pH-cycling models using bovine enamel. Caries Res. 2005;39(6):514-20. doi: 10.1159/000088189

20. Chedid SJ, Cury JA. Effect of 0.02% NaF solution on enamel demineralization and fluoride uptake by deciduous teeth in vitro. Brazilian oral research. 2004;18(1):18-22. doi: 10.1590/S1806-83242004000100004

21. Attin T, Manolakis A, Buchalla W, Hannig C. Influence of tea on intrinsic colour of previously bleached enamel. J Oral Rehabil. 2003;30(5):488-94.

22. Coutinho GG, Goloni-Bertollo EM, Pavarino-Bertelli EC. Effectiveness of two programs of intermittent ferrous supplementation for treating iron-deficiency anemia in infants: randomized clinical trial. Sao Paulo Med J. 2008;126(6):314-8. doi: 10.1590/S1516-31802008000600004

23. Watts A, Addy M. Tooth discolouration and staining: a review of the literature. Br Dent J. 2001;190(6):309-16. doi: 10.1038/sj.bdj.4800959a

24. Clarke M, Locker D, Berall G, Pencharz P, Kenny DJ, Judd P. Malnourishment in a population of young children with severe early childhood caries. Pediatr Dent. 2006;28(3):254-9.

25. Hadler MC, Sigulem DM, Alves MFC, Torres VM. Treatment and prevention of anemia with ferrous sulfate plus folic acid in children attending daycare centers in Goiania, Goias State, Brazil: a randomized controlled trial. Cad Saude Publica. 2008;24 Suppl 2:S259-71.