In Vitro Efficacy of Sodium Bicarbonate Powder and Pumice Flour for Removal of Iron Induced Pigmentations

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Abstract

Background and Aim: Management of iron-induced tooth pigmentation is challenging. This study aimed to compare the efficacy of sodium bicarbonate powder and pumice flour for removal of iron-induced pigmentation from primary anterior teeth.

Materials and Methods: In this in vitro, experimental study, 60 sound primary anterior teeth were collected. After cutting the roots and sealing the pulp chamber with composite resin, all teeth were coated with acid gel for 30 s and then immersed in 250 cc of ferrous sulfate iron drop for 48 h to induce iron discoloration. Afterwards, the teeth were randomly divided into two experimental groups (n=30). In group A, discolored teeth were cleaned with pumice/water slurry. Samples in group B were cleaned similarly for 30 s using sodium bicarbonate/water slurry. The shade of all teeth was evaluated at baseline, after inducing iron staining and following stain removal using Vita Easy Shade spectrophotometer. The data were analyzed using Kolmogorov-Smirnov test, t-test and ANCOVA. P<0.05 was considered statistically significant.

Results: All teeth showed color improvement following the cleaning procedures. However, teeth in pumice group showed a significantly greater mean Δ E2 value compared with sodium bicarbonate, representing more efficient stain removal (P=0.926 for L, P=0.162 for a*, P=0.980 for b*).

Conclusion: Both sodium bicarbonate and pumice were effective for tooth stain removal, although pumice powder showed significantly higher efficacy. **Key Words:** Tooth Discoloration, Iron, Sodium Bicarbonate, Pumice

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Introduction

Iron deficiency is the most prevalent nutritional deficiency in the world, with significant negative health consequences including poor physical, cognitive and behavioral development of the affected patients, particularly children (1). Iron salts in the form of oral iron drops are often prescribed for preschool children to prevent or treat iron deficiency and anemia in this vulnerable age group (1,2). Despite the importance of iron supplementation in

iron-deficient populations, irregular or inadequate iron intake is reported due to various reasons, such as dental esthetic reasons since iron intake induces staining (3).

Tooth color in the maxillary anterior region is of great importance to children and their parents, since it is considered as a key factor in dental esthetics (4,5). Children as young as 3 years of age are capable of differentiating between attractive and unattractive appearance of their peers' smile. Thus, tooth pigmentation may

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adversely affect the perception of facial esthetics and consequently self-confidence and social interaction of preschool children (3,6).

Tooth discoloration may be due to intrinsic or extrinsic staining according to the origin and location of the stains. Superficial staining occurs in the acquired pellicle on the tooth surface. It is promoted by external chromogens such as foods and beverages, metal salts or cationic antiseptic agents and may be classified as direct (producing stains according to the basic color of the stain source) or indirect (due to chemical reaction on the tooth surface) with a metallic or non-metallic origin (6-9). There is ample literature on the incidence of extrinsic black discoloration on the teeth of children consuming supplements (3,8). Iron may interact with polyphenolic dietary chromogens or hydrogen sulfide produced by microflora exudated in the saliva or gingival crevicular fluid (3,7). Black iron staining on primary teeth is a cause of concern to parents as they are concerned about their child's tooth appearance and seek dental care for tooth color improvement (3).

Management of tooth discoloration includes polishing and prophylaxis with an abrasive paste, micro- and macro-abrasion, bleaching and veneers or full crowns (8,10). Some methods such as micro- and macro-abrasion may grossly improve the tooth color; however, the inherent danger of using high-concentration powerful acids during the micro-abrasion procedure or irreversible enamel loss during macro-abrasion method have limited their application for young patients (11,12). Thus, removal of extrinsic tooth staining is commonly confined to mechanical tooth cleaning with abrasive pastes, as the chromogens are localized on the tooth surface within the acquired pellicle (13), unless internalized by enamel defects (7). Mechanical approaches include tooth brushing, professional tooth cleaning, ultrasonic and sonic scaling, selective polishing and air jet polishing (8,13). Currently, professional application of abrasives to remove the stains is the most accepted approach to abolish the surface pigmentations (13).

Pumice is one of the commonly used abrasive

products for professional tooth cleaning, which is available in 2 forms: pumice powder and paste. The abrasive particle sizes vary between different commercial brands differing from fine to coarse, according to the sieve through which the particles pass. It is stated that prophylaxis with pumice removes the protective surface enamel, leaving a rough surface susceptible to further staining (8, 14-16).

Sodium bicarbonate, also known as baking soda, is widely used for nearly 100 years as an abrasive in a variety of cleaning products. Its stain and plaque removal ability, despite its low abrasivity, suggest chemical as well as physical mechanisms for its stain removal action leading to more cleaning power with minimal abrasion (17,18). Low tooth structure abrasion, safety, biocompatibility, low cost and buffering and antibacterial properties made it one of the most economical, yet efficient, abrasive particularly for use in dental products such as dentifrices and mouth rinses (17-20). It has shown 10 times more abrasion-cleaning power than calcium phosphate as the gold standard for mechanical cleaning (18).

Following stain removal techniques, tooth color alterations are assessed by several methods, including the use of shade guides and colorimeters, and spectrophotometry. Various researchers have shown that spectrophotometry is the most accurate measurement tool for assessment of tooth color change (3,9).

Considering the proven benefits of sodium bicarbonate in dentifrices for stain removal, the aim of this in vitro study was to compare the efficacy of sodium bicarbonate powder and pumice flour in removing iron-induced pigmentations from primary anterior teeth as a professional tooth cleaning material.

Materials and Methods

Sixty primary anterior teeth (central and lateral incisors) extracted within 1 month prior to the study onset due to pre-shedding mobility were selected and sterilized by 10% formalin for 24 hours. They were stored in distilled water, which was refreshed weekly to minimize and kept hydrated contamination were throughout the experiment. Teeth with restorations, developmental defects, stains (intrinsic or extrinsic) and cracks were excluded. The study was approved by the ethics committee of our university (IR Shahed.REC.1395.182).

A low-speed hand-piece was used to clean the tooth surface and a stereomicroscope (K450X; Emitech, London, UK) was used at x40 magnification to exclude defective teeth. The roots were cut by a low-speed diamond saw (Isomet, Buhler LTD, lake bluff, IL, USA), and the pulp tissue was removed from the pulp chamber using #35 and #40 K-files (Mani Inc., Tochigi, Japan). The pulp chambers were restored with solid A1 shade of Z250 composite resin (3M, ESPE, St Paul, MN, USA). Following this procedure, the samples were horizontally mounted in self-cure acrylic resin with the buccal surface facing upward and exposed. Next, they were randomly divided into two experimental groups of A and B (n=30). *Tooth staining:*

Specimens in both groups were coated with etchant gel (Scotchbond: 3M ESPE St Paul MN

etchant gel (Scotchbond; 3M ESPE, St Paul, MN, USA) for 30 s in order to obtain a rough surface with enamel porosities, increased surface area and subsequently maximum extrinsic discoloration (19,21). The teeth in each group were immersed in 250 mL of iron drop (Kharazmi Co., Tehran, Iran) for 48 h and rinsed to remove the excess iron solution afterwards. Next, shade assessment was performed.

Correction of tooth discoloration:

In group A, the discolored labial surface of the teeth was cleaned by a mixture of 3 cc of water added to 3 g of medium-grit pumice powder (Kimia Corp., Tehran, Iran) to make a water slurry of pumice flour. Samples in group B were cleaned similarly by a water slurry of sodium bicarbonate by mixing 3 g of baking soda (Golha Corp., Tehran, Iran) with 3 cc of distilled water. Both pastes were applied and rubbed on the buccal surface of the teeth using a rubber cup on a contra-angle slow-speed hand-piece (10:1) for 30 s by a blinded operator, with no added hand pressure. Before the shade assessment, the specimens were rinsed with distilled water to remove the paste remaining on the tooth surface.

Shade assessment:

The shade of all samples was evaluated at baseline (phase 1), after induction of iron staining (phase 2) and following stain removal (phase 3) using Compact Vita Easy Shade spectrophotometer (Vita, Bad Säckingen, Germany) according to the CIE L*a*b* color system. The color change between phases 1 and 2 and between phases 2 and 3 was reported as Δ E1 and Δ E2, respectively. Δ E was measured using the color difference formula as follows (4): Δ E = $[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$

L* is the degree of lightness ranging from 0 (absolute black) to 100 (reference white). The a* coordinate indicates the red (positive a*) and green (negative a*) axis while the b* coordinate indicates the yellow (positive b*) and blue (negative b*) axis (4,22).

The instrument was calibrated according to the manufacturer's instructions before examination. During the shade assessment, the probe tip was in direct contact with the tooth surface.

The difference in color change among different phases was analyzed using the Kolmogorov-Smirnov test followed by t-test and ANCOVA. P<0.05 was considered statistically significant.

Results

Table 1 demonstrates the mean baseline L*, a* and b* values of the experimental groups with no significant difference between them (P=0.926 for L*, P=0.162 for a* and P=0.980 for b*).

The mean L*, a* and b* values for the teeth following staining and cleaning procedures are presented in Tables 2 and 3, respectively. According to Tables 1 and 2, the stained teeth showed decreased L* value, positive a* value and increased b* value compared with baseline measurements, which indicated a shift to darker teeth with the direction of red and yellow, with no significant difference between the two groups (P>0.05).

Comparison of the L*, a* and b* values in Tables 2 and 3 revealed an increase in L* value, and a recovery of a* and b* values of stained teeth towards green and blue after polishing, although the three coordinates were not

	Mean (± standard deviation)		
Parameter	Sodium bicarbonate	Pumice	P value
	(n=30)	(n=30)	
L*	90.43(±3.8)	90.35(±3.2)	0.926
a*	-1.01(±0.49)	-0.82(±0.53)	0.165
b *	20.93(±4.3)	20.95(±3.6)	0.980

Table 1. Baseline mean L*, a* and b* values of teeth in the two groups

Table 2. Mean L*, a* and b* values of teeth in the two groups following stain formation

	Mean (± standard deviation)		
Parameter	Sodium bicarbonate (n=30)	Pumice (n=30)	P value
L*	73.14(±2.6)	72.65(±2.4)	0.455
a*	2.91(±0.48)	3.11(±0.53)	0.128
b*	31.53(±2.8)	31.35(±3.0)	0.810

Table 3. Mean L*, a* and b* values of teeth in the two groups following treatmentwith the polishing agent

	Mean (± standard deviation)		
Parameter	Sodium bicarbonate	Pumice	
	(n=30)	(n=30)	
L*	76.87(±3.03)	77.43(±2.33)	
a*	0.99(±0.83)	0.887(±0.74)	
b *	27.06(±2.8)	25.68(±2.5)	

completely reversed to the baseline values mentioned in Table 1.

The mean overall color change values after discoloration (Δ E1) and following cleaning (Δ E2) are shown in Table 4. There was a significant difference between the two polishing

treatments (pumice and sodium bicarbonate) according to $\Delta E2$ values. The specimens in pumice group showed a greater mean $\Delta E2$ value compared with sodium bicarbonate group, representing a more efficient stain removal by pumice (P=0.009).

	Mean (± standard deviation)		
Parameter	Sodium bicarbonate	Pumice	P value*
	(n=30)	(n=30)	
ΔΕ1†	20.98(±3.4)	21.19(±2.7)	-
ΔE2‡	6.8(±1.9)	8.3(±2.1)	0.009
*: ANCOVA			

Table 4. Color change (ΔE) following stain formation	on and stain removal
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†: Overall color change following discoloration
 ‡: Overall color change following stain removal

Discussion

Extrinsic tooth staining following iron supplementation is reported to be the cause of concern for many parents, as half of the children receiving iron supplements demonstrate tooth pigmentation (2,3,6). Different methods and products are suggested to remove superficial iron staining using professionally applied abrasives (13,19). However, the abrasive capacity and stain removing efficacy of the polishing pastes and powders vary greatly, which may result in diverse tooth wear and cleaning power (19,23). In the present study, we compared the iron induced stain removing potential of polishing with pumice powder for 30 s as a traditional in-office polishing material with a solely mechanical action, and sodium bicarbonate as a product acting both mechanically and chemically on the discolored labial surface of primary anterior teeth (13,17). We used spectrophotometry to assess the tooth color and its alterations following discoloration and stain removal. There are various methods to evaluate the tooth color including visual subjective judgement by the shade guides or objective methods such as colorimetry or spectrophotometry (5,9). Shade matching, although commonly used in everyday practice, is highly reliant on individual variations, and its accuracy is dependent on the operator's experience. Disagreements have been reported between the operator's judgement and the actual tooth color, even for the same specimen and the same operator at different times (2,24). Despite the objectivity of colorimetry compared with shade guides, it is prone to significant edge-loss effect and systematic errors. Also, poor reliability of this instrument has limited its

usage in dentistry (5,22). Unlike colorimeters perform the measurements in that 3 wavelengths (red, green, blue), spectrophotometers evaluate the reflectance of light within the entire visible spectrum, which makes it a reference tool for color analysis of convex and asymmetric objects like teeth (5,22,25). Tooth color is the result of the incident light behavior on the tooth surface including scattering and absorption. The darker the tooth color, the greater the light absorption by the tooth and the lower the amount of reflected light would be, which is perceived by spectrophotometers (5). Following light emitting, the reflected light will be converted into digital codes by the device and demonstrated in L* (lightness), a* (redgreen) and b* (yellow-blue) coordinates in the CIE L*a*b* color space. Afterwards, the overall color change (ΔE) is calculated according to the aforementioned three coordinates (3,5,13,22). Our results showed the mean baseline values of 90.3, -.0.9 and, 20.9 for L*, a*and b*, respectively. Hasegawa et al. (26) reported the mean values of L* (73.0), a* (3.5) and b* (16.5) for permanent teeth in a Japanese population. Comparing with our results for primary teeth, their findings showed darker, redder and bluer shades for permanent teeth. In an investigation conducted by Kim et al, (27) the mean L*, a* and b* values for primary teeth were 82.5, 0.2, 18.3, respectively. Their findings were close to our results with little differences, which may be various methods and due to samples implemented in the two studies. In contrast to our in vitro study on primary anterior teeth, they evaluated the color of all primary teeth in a clinical condition and different background light.

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When comparing the reflectance values of the specimens following staining in our study, lower reflectance values were recorded by the reflectance spectrophotometer compared with the baseline values, demonstrating darker tooth color. This confirms that a discoloration occurred during the stain development procedure. Subsequent to stain removal phase, a greater reflectance in comparison with the pigmentation phase was detected, showing optimal efficacy of the cleaning procedure. We found $\Delta E1$ of 20.9 and 21.1 following stain formation in the two experimental groups, which were far above the clinical visibility threshold ($\Delta E=2.7$); this was also observed after the whitening procedure ($\Delta E2=6.8$ and 8.3) (4). In the present study, both treatment protocols resulted in improvement of iron simulated discoloration, although pumice flour was more effective. The stain removal action of pumice is solely because of its abrasivity by a mechanical function as a result of its particles' high hardness value. The effectiveness of the abrasive material is shown by its hardness, as measured by the Mohs hardness value of the agent. Pumice with a Mohs hardness number of 6-7 compared with enamel (with a Mohs hardness number of 5) is capable of enamel wear, which leads to mechanical stain cleaning and at the same time tooth structure removal (18,23). According to a previous study, polishing with pumice for 30 s can remove 0.6 μm to 4 μm of the outer enamel surface, containing the highest fluoride concentration and create a rough surface contributing to further harboring of bacteria and external discolorations (15). Nevertheless, an ideal abrasive should provide stain removal without causing significant tooth wear (5). Our results demonstrated an acceptable stain removal capacity for sodium bicarbonate, although lower than pumice. Sodium bicarbonate, as a well-known abrasive and whitening agent implemented in many commercial dentifrices, demonstrates a superior cleaning power compared with abrasives with almost similar or greater hardness values (Mohs hardness number of 2.5) (17,18). The low tooth structure removal and high cleaning power of sodium

bicarbonate are explained by its good water solubility and its interference with plaque formation decreasing the count by of pathogenic microbial oral flora and attenuating the critical pH. Unlike other abrasives such as silica and aluminum oxide, which are chemically perform inert and only mechanical debridement, sodium bicarbonate has mechanical and biological modes of action, increasing its efficacy (18). In addition, sodium bicarbonate is bacteriostatic, which may prevent discolorations and caries caused by oral chromogenic and cariogenic bacteria (19). Although our results demonstrated a color improvement in both groups after polishing, none of the experimental groups exhibited a

stain recovery close to baseline color prior to stain development, confirming that total extrinsic stain removal is difficult by common prophylactic measures (13,19). This may be explained by the fact that surface pigmentation may permeate into the underlying tooth structure, becoming internalized (9,7), which may not be removed solely by mechanical action of abrasives (7). Thus, the cleaning power of polishing material is more important than its abrasivity (19). This is particularly evident in whitening dentifrices. A previous study stated that complete stain removal needs decolorizing agents like hydrochloride acid or hydrogen peroxide, which are destructive to tooth structure and difficult to use in young children with poor cooperation (13).

In this study, we used ferrous sulfate iron drops containing 25 mg of iron per dose (1 mL), which is consistent with the literature regarding the ideal dose (12.5-50 mg) of iron supplementation (3). Formulations with lower iron content may produce less tooth staining, but will not prevent anemia (3, 28,29).

Although extrinsic tooth staining is mainly caused by the interaction of metal ions and anionic external chromogens on the tooth surface, enamel defects or porosities exaggerate the intensity of staining by attracting the extrinsic stains (7,9,19). There are several stain inducing procedures used to cause the pigmentations, some of them are time demanding or leading to inadequate discoloration (30). Previous clinical trials indicated that insufficient stain development is one of the reasons in reporting failure to show extrinsic stain inhibition and removal by stain cleansing agents (3,31). To standardize the initial tooth pigmentation, we used an acid etch gel to develop porous enamel and increase the surface area to optimize extrinsic stain formation. Following induction of tooth staining, the spectrophotometer disclosed a similarity in discoloration of specimens in both groups and a clinically visible color change compared with the initial tooth color according to Δ E1 values (Δ E1 >2.7), demonstrating the optimal efficacy of pigmentation protocol (4).

We used a Vita Easy Shade spectrophotometer, which is a hand-held spectrophotometer with its own light source. The smaller size of primary teeth compared with permanent teeth raises a potential concern about the accuracy of color measurements. Although we used primary anterior teeth with larger labial surface area, the diameter of the inner ring of the probe was 5 mm, with 2 spectrometers placed in a diameter not exceeding 3 mm within the inner ring, which compensates the small dimensions of primary teeth. Correct freehand positioning of the device is also important in preventing errors. This is controlled by 3 fibers installed in the probes' inner ring to prevent any color readings, if the perpendicular position of the probe is not fulfilled (27).

Although in vitro investigations are of great importance in evaluating the stain removal efficacy of dental materials and procedures (9), in vivo and in vitro conditions may differ in many ways, as dental pellicle and oral bacteria have a significant role in development and adhesion of discolorations and thus their removal. On the other hand, sodium bicarbonate is one of the most multifunctional abrasives available today, with the ability of caries prevention, anti-tartar activity and reduction in plaque formation and adhesion, which may be mainly assessed in clinical situations (18). Accordingly, we suggest further in vivo or in situ studies to evaluate the efficacy of sodium bicarbonate for prevention and removal of clinically developed iron stains.

Conclusion

Within the limitations of this in vitro study, the results showed that both sodium bicarbonate and pumice were effective for tooth stain removal, although pumice powder was significantly more efficient.

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References

1. Murray-Kolb LE, Beard JL. Iron deficiency and child and maternal health. Am J Clin Nutr. 2009 Jan;89(3):946S-950S.

2. De-Regil LM, Jefferds ME, Sylvetsky AC, Dowswell T. intermittent iron supplementation for improving nutrition and development in children under 12 years of age. Cochrane Database Syst Rev. 2011 Dec 7;(12):CD009085.

3. Pani SC, Alenazi FM, Alotain AM, Alanazi HD, Alasmari AS. Extrinsic tooth staining potential of high dose and sustained release iron syrups on primary teeth. BMC Oral Health. 2015 Aug 4; 15:90.

4. Paravina RD, Majkic G, Stalker JR, Kiat-Amnuay S, Chen JW. Development of a model shade guide for primary teeth. Eur Arch Paediatr Dent. 2008 June;9(2):74-78.

5. Lima DA, Silva AL, Aguiar FH, Liporoni PC, Munin E, Ambrosano GM, et al. In vitro assessment of the effectiveness of whitening dentifrices for the removal of extrinsic tooth stains. Braz Oral Res. 2008 Apr-Jun;22(2):106-11. 6. Kumar A, Kumar V, Singh J, Hooda A, Dutta S. Drug-induced discoloration of teeth: an updated review. Clin Pediatr (Phila). 2012 Feb;51(2): 181-5.

Prathap S, Rajesh H, Boloor VA, Rao AS.
 Extrinsic stains and management: A new insight. J Acad Indus Res. 2013 Jan;1(8):435-442.
 ST Manuel, P Abhishek, M Kundabala.
 Etiology of tooth discoloration- a review. Nig Dent J. 2010;18(2):56-63.

9. Lee BS, Huang SH, Chiang YC, Chien YS, Mou CY, Lin CP. Development of in vitro tooth stai; ning model and usage of catalysts to elevate the

effectiveness of tooth bleaching. Dent Mater. 2008 Jan;24(1):57-66.

10. Walsh TF, Rawlinson A, Wildgoose D, Marlow I, Haywood J, Ward JM. Clinical evaluation of the stain removing ability of a whitening dentifrice and stain controlling system. J Dent. 2005 May;33(5):413-8.

11. Loguercio AD, Correia LD, Zago C, Tagliari D, Neumann E, Gomes OM, et al. Clinical effectiveness of two microabrasion materials for the removal of enamel fluorosis stains. Oper Dent. 2007 Nov-Dec;32(6):531-8.

12. Heymann H, Swift EJ, Ritter A. Sturdevant's Art and Science of Operative Dentistry. 6th ed., 2013, Mosby, St Louis: pp:300-2.

13. Nathoo SA, Gaffar A. Studies on Dental Stains Induced by Antibacterial Agents and Rational Approaches for Bleaching Dental Stains. Adv Dent Res. 1995 Dec;9(4):462-70.

14. Honório HM, Rios D, Abdo RC, Machado MA. Effect of different prophylaxis methods on sound and demineralized enamel. J Appl Oral Sci. 2006 Apr;14(2):117-23.

15. American Academy of Pediatric Dentistry Clinical Affairs Committee; American Academy on Pediatric Dentistry Council on Clinical Affairs. Guideline on the role dental prophylaxis in pediatric dentistry. Pediatr Dent. 2008-2009; 30(7 Suppl):119-20.

16. Watts A, Addy M. tooth color discoloration and staining: A review of literature. Br Dent J. 2001 Apr;190(6):309-16.

17. Ghassemi A, Hooper W, Vorwerk L, Domke T, DeSciscio P, Nathoo S. Effectiveness of a new dentifrice with baking soda and peroxide in removing extrinsic stain and whitening teeth. J Clin Dent. 2012;23(3):86-91.

18. Myneni SR. Effect of baking soda in dentifrices on plaque removal. J Am Dent Assoc. 2017 Nov;148(11S):S4-S9.

19. Li Y. Stain removal and whitening by baking soda dentifrice: A review of literature. J Am Dent Assoc. 2017 Nov;148(11S):S20-S26.

20. Silhacek KJ, Taake KR. Sodium bicarbonate and hydrogen peroxide: the effect on the growth

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of Streptococcus mutans. J Dent Hyg. 2005 Fall; 79(4):7.

21. Cerci BB, Roman LS, Guariza-Filho O, Camargo ES, Tanaka OM. Dental enamel roughness with different acid etching times: Atomic force microscopy study. Eur J Gen Dent 2012 Sep;1(3):187-91.

22. Joiner A, Hopkinson I, Deng Y, Westland S. A review of tooth color and whiteness. J Dent. 2008;36 Suppl 1:S2-7.

23. Wilkins EM. Clinical practice of dental hygienist.12th ed. Philadelphi: Lippincott Williams and Wilkins: 2016; 730-42.

24. Macpherson LM, Stephen KW, Joiner A, Schäfer F, Huntington E. Comparison of a conventional and modified tooth stain index. J Clin Periodontal 2000 Nov; 27(11):854-9.

25. Usumez A, Ozturk N, Ozturk B. Two-year color changes of light-cured composites: influence of different light-curing units. Oper Dent. 2005 Sep-Oct;30(5):655-60.

26. Hasegawa A, Motonomi A, Ikeda I, Kawaguchi S. Color of natural tooth crown in Japanese people. Color Res Appl. 2000 Feb; 25 (1):43-8.

27. Kim J, Paravina R, Chen JW. In vivo evaluation of color of primary teeth. Pediatr Dent. 2007 Sep-Oct;29(5):383-6.

28. Christofides A, Asante KP, Schauer C, Sharieff W, Owusu-Agyei S, Zlotkin S. Multimicronutrient sprinkles including a low dose of iron provided as microencapsulated ferrous fumarate improves haematologic indices in anaemic children: a randomized clinical trial. Matern Child Nutr. 2006 Jul;2(3):169-80.

29. Griffin IJ, Cooke RJ, Reid MM, McCormick KP, Smith JS. Iron nutritional status in preterm infants fed formulas fortified with iron. Arch Dis Child Fetal Neonatal Ed. 1999 Jul;81(1):F45-9.

30. Pontefract H, Courtney M, Smith S, Newcombe RG, Addy M. Development of methods to enhance extrinsic tooth discoloration for comparison of toothpastes. 1. Studies in vitro. J Clin Periodontol. 2004 Jan; 31 (1):1-6.