Isabelle Rodrigues Freire

EFEITO DE DENTIFRÍCIOS COM CONCENTRAÇÃO REDUZIDA DE FLUORETO SUPLEMENTADOS COM POLIFOSFATOS NA INCIDÊNCIA DE LESÕES DE CÁRIE DENTÁRIA E NA INGESTÃO DE FLUORETO: ESTUDO CLÍNICO LONGITUDINAL EM CRIANÇAS.

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Tese apresentada à Faculdade de Odontologia, Campus de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho" como parte dos requisitos para obtenção do título de Doutorado em Ciência Odontológica
(Área de concentração: Saúde Bucal da Criança).

Orientador: Prof. Dr. Alberto Carlos Botazzo Delbem

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Dedicatória

Dedico este trabalho,

(in memoriam)

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"Hoje levantei cedo pensando no que tenho a fazer antes que o relógio marque meia noite. É minha função escolher que tipo de dia vou ter hoje. Posso reclamar porque está chovendo ou agradecer às águas por lavarem a poluição. Posso ficar triste por não ter dinheiro ou me sentir encorajado para administra minhas finanças, evitando o desperdício. Posso reclamar sobre minha saúde ou dar graças por estar vivo. Posso me queixar dos meus pais por não terem me dado tudo o que eu queria ou posso ser grato por ter nascido. Posso reclamar por ter que ir trabalhar ou agradecer por ter trabalho. Posso sentir tédio com o trabalho doméstico ou agradecer a Deus por ter um teto para morar. Posso lamentar decepções com amigos ou me entusiasmar com a possibilidade de fazer novas amizades. Se as coisas não saíram como planejei posso ficar feliz por ter hoje para recomeçar. O dia está na minha frente esperando para ser o que eu quiser. E aqui estou eu, o escultor que pode dar forma. Tudo depende só de mim."

(Charles Chaplin)

Resumo

FREIRE, IR. *Efeito de dentifrícios com concentração reduzida de fluoreto suplementados com polifosfatos na cárie dentária e na ingestão de fluoreto: estudo clínico longitudinal em crianças.*Tese de Doutorado em Ciência Odontológica (Saúde Bucal da Criança) – Faculdade de Odontologia, Campus de Araçatuba, Universidade Estadual Paulista “Júlio de Mesquita Filho”, 2013.

Resumo

O objetivo do presente estudo foi avaliar (1) o efeito anticárie de dentifrícios com concentração reduzida de fluoreto (DCRFs) suplementados com polifosfatos e (2) a concentração de fluoreto (F) nas unhas das mãos e pés de bebês após o uso de DCRFs. No primeiro estudo, crianças provenientes de creches dos municípios de Araçatuba e Fernandópolis (Estado de São Paulo), com idade média de 48 meses, foram aleatoriamente divididas em três grupos, de acordo com os dentifrícios a serem utilizados (protocolo duplo-cego): 500 ppm F com 1% de trimetafosfato de sódio (“500 TMP”, *n*=206), 500 ppm F com 0,25% de glicerofosfato de cálcio (“500 GPCa”, *n*=193) e 1100 ppm F (*n*=201). Exames clínicos (ceo-s) foram realizados no início e aos 18 meses do estudo, e o incremento de lesões de cárie (IC) foi calculado para crianças com e sem experiência prévia de cárie (EC). Para o segundo estudo, crianças (18 a 30 meses de idade, *n*=56) sem uso prévio de dentifrícios fluoretados, provenientes de um programa preventivo para bebês, fizeram uso dos dentifrícios supramencionados. Unhas das mãos e pés foram coletadas mensalmente, durante 12 meses, a partir do início do uso dos dentifrícios. O F ingerido a partir da água, dieta e dentifrício também foram determinados. As análises foram realizadas com um eletrodo íon-seletivo, após difusão facilitada por HMDS ou pelo método direto, de acordo com as amostras. Os dados foram submetidos a ANOVA a 1 critério (ingestão de F pela água) e 2 critérios (IC, concentração de F nas unhas e ingestão de F pela dieta), seguida do teste de Student-Newman-Keuls ($p < 0,05$). O IC foi significativamente menor com o uso do dentifrício 500 TMP ($p < 0,05$), tendo os dentifrícios 1100 ppm F e 500 GPCa apresentado IC similares ($p=0,275$). Não houve diferenças significativas entre os dentifrícios para ceo-s inicial, ceo-s final e IC nas crianças sem EC. Nas crianças com EC, o dentifrícios 500 TMP e 500 GPCa apresentaram menores valores de IC quando comparado ao 1100 ppm F ($p < 0,05$). Os níveis de F nas unhas das mãos e pés, bem como ingestão de F do dentifrício foram semelhantes para os grupos tratados com 500 TMP e 500 GPCa, mas significativamente menores quando comparados ao dentifrício 1100 ppm F ($p<0,05$).

Não foram observadas diferenças significativas entre os grupos em relação à ingestão de F a partir da dieta e água (p>0,05). Concluiu-se que os DCRFs suplementados com polifosfatos apresentaram eficácia anticárie similar ou superior à de um dentifrício com 1100 ppm F, ao mesmo tempo em que levaram a uma menor ingestão e absorção de F em comparação ao dentifrício padrão. De acordo com o protocolo do estudo, as unhas podem ser consideradas como biomarcadores de exposição ao F por dentifrícios com diferentes concentrações de fluoreto na faixa etária estudada.

Descritores: Cárie Dentária. Dentifrícios. Fluoretos. Polifosfatos. Unhas. Crianças.

FREIRE, IR. *Effect of low-fluoride dentifrices supplemented with polyphosphates on dental caries and fluoride intake: longitudinal clinical study.*Tese Doctor Dental Science-Child Health– Dental School of Araçatuba, São Paulo State University “Júlio de Mesquita Filho”, 2013.

ABSTRACT

The aim of the present study was to assess (1) the anticaries effect of low-fluoride dentifrices (LFDs) supplemented with polyphosphates and (2) fluoride (F) concentrations in fingernails and toenails of babies after the use of LFDs. In the first study, children attending day care centers from the cities of Araçatuba and Fernandópolis (State of São Paulo, Brazil), with average age of 48 months, were randomly assigned to 3 different groups (double-blind protocol), according to the dentifrices to be used: 500 ppm F with sodium trimetaphosphate (“500 TMP”, *n*=206), 500 ppm F with 0,25% calcium glycerophosphate (“500 CaGP”, *n*=193) and 1100 ppm F (*n*=201). Clinical examinations (dmfs) were done at the beginning and 18 months after using the dentifrices, and caries increment (CI) was calculated for children with and without previous caries experience (CE). For the second study, children (18 to 30 months old, *n*=56), without previous contact with fluoridated dentifrices and who attended a preventive program directed to babies, were randomly assigned to the above-mentioned dentifrices (double-blind protocol). Fingernails and toenails were monthly clipped from the beginning of the study for up to 12 months. F intake from water, diet and dentifrice was also determined. F analyses were carried out with an ion-selective electrode after HMDS-facilitated diffusion or by the direct method, depending on the sample. Data were submitted to 1-way (F intake from water) or 2-way (CI, F concentrations in nails and F intake from the diet) ANOVA, followed by Student-Newman-Keuls test ($p<0.05$). CI was significantly lower for children using the 500 TMP dentifrice ($p<0.05$), with similar values for the 1100 ppm F and 500 CaGP dentifrices ($p=0.275$). No significant differences were found among the dentifrices for initial and final dmfs, and for CI for children without CE. For children with CE, significantly lower CI was obtained for the groups 500 TMP and 500 CaGP ($p<0.05$). F levels in fingernails and toenails were similar for children using 500 TMP and 500 CaGP, but significantly lower when compared to the 1100 ppm F dentifrice ($p<0.05$). No significant differences were found among the groups regarding F intake from the diet and water ($p>0.05$). It was concluded that LFDs supplemented with polyphosphates presented similar or

superior anticaries effect when compared to the 1100 ppm F, at the same time as they promoted lower F intake and absorption compared to the standard dentifrice. According to the study protocol, nails can be considered as biomarkers of exposure to F from dentifrices with different F concentrations in the studied age range.

Descriptors: Dental Caries. Dentifrices. Fluoride. Polyphosphates. Nails. Children.

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Introdução Geral

1- Introdução Geral

Nas últimas décadas, tem sido observado um declínio expressivo na prevalência da cárie dentária em diversas partes do mundo, o qual tem sido atribuído principalmente à fluoretação da água de abastecimento e ao uso de dentifrícios fluoretados [1]. O uso difundido dos dentifrícios, entretanto, tem sido associado a um aumento da prevalência de fluorose dentária, especialmente quando dentifrícios contendo 1000 ppm F ou mais são utilizados por crianças com idade abaixo de 5-6 anos [2].

Alternativas para se reduzir a ingestão de fluoreto e, conseqüentemente, minimizar o risco de desenvolvimento da fluorose dental, incluem a redução da quantidade de dentifrício colocada na escova, bem como a supervisão da criança durante a escovação quanto à quantidade de dentifrício usada e expectoração deste após a escovação. Uma vez que tais medidas dependem primariamente da adesão da criança e seus responsáveis às mesmas, existe a possibilidade do não seguimento das recomendações do profissional. Assim, tem sido proposto o uso de dentifrícios com concentração reduzida de fluoreto (DCRF) por crianças abaixo dos 6 anos de idade [3,4].

Entretanto, visto que ainda não existe consenso na literatura quanto à eficácia anticárie destes quando comparada à de dentifrícios com concentração convencional de fluoreto (1000-1100 ppm), alternativas como a redução do pH dos

[1] Bowen WM. Fluorosis is it really a problem? *J Am Dent Assoc* 2002;133: 1405-7.
[2] Wong MCM, Glenny AM, Tsang BWK, Lo ECM, Worthington HV, Marinho VCC: Topical fluoride as a cause of dental fluorosis in children. Cochrane Database Syst Rev 2010;1: CD007693.
[3] Vilhena FV, Silva HM, Peres SH, Caldana Mde L,Buzalaf MA: The drop technique: a method to control the amount of fluoride dentifrice used by young children. Oral Health Prev Dent 2008; 6: 61‑65.
[4] Pessan JP, Toumba KJ, Buzalaf MA. Topical use of fluorides for caries control. *Monogr Oral Sci* 2011; 22: 115–132.

DCRFs [5,6,7,8,9,10] ou a suplementação destes com sais de polifosfato [11,12,13] têm levado a resultados semelhantes ou até mesmo superiores aos de formulações convencionais.

Recentemente foi demonstrado *in vitro* que a adição de trimetafosfato de sódio (TMP) a um DCRF (500 ppm F) promoveu uma menor perda mineral do esmalte em comparação a um dentifrício convencional (1100 ppm F)[11,12]. Semelhantemente, a adição de 0,25% de glicerofosfato de sódio (GPCa) a um DCRF produziu um efeito anticárie similar ao de um dentifrício com 1100 ppm F em um estudo *in situ* [13]. Como infantes usualmente ingerem grande quantidades de dentifrícios durante a escovação dental [14],[15],[16] e considerando que somente o fluoreto absorvido tem impacto no desenvolvimento da fluorose dentária[17], seria

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[5] Negri HMD, Cury JA: Dose-response effect of a dentifrice formulation with low fl uoride concentration: An in vitro study. Pesq Odontol Bras 2002; 16: 361–365.

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[10] Vilhena FV, Olympio KPK, Lauris LRP, Delbem ACB, Buzalaf MAR. Low-Fluoride acidic dentifrice: a randomized clinical trial in a fluoridated area. Caries Res 2010; 44: 478-484.

[11] Takeshita E M, Castro LP, Sassaki K T, Delbem ACB. In vitro evaluation of dentifrice with low fluoride content supplemented with trimetaphosphate. *Caries Res.* 2009; 43: 50-56.

[12] Takeshita EM, Exterkate RAM, Delbem ACB, ten Cate JM. Evaluation of different fluoride concentrations supplemented with trimetaphosphate on enamel de- and remineralization in vitro. Caries Res 2011; 45: 494–497.

[13] Amaral JG, Sassaki KT, Martinhon CCR, Delbem ACB. Effect of low-fluoride dentifrices supplemented with calcium glycerophosphate on enamel demineralization in situ. Am J Dent 2013;26:000-000. (In Press).

[14] Lima YBO, Cury JA. Fluoride intake by children from water and dentifrice. Rev Saúde Pública 2001; 35:576-81.

[15] De Almeida BS, da Silva Cardoso VE, Buzalaf MAR. Fluoride ingestion from toothpaste and diet in 1- to 3-year-old Brazilian children. Community Dent Oral Epidemiol 2007; 35:53-63.

[16] Kobayashi CA, Belini MR, Italiani FD, Pauleto AR, Julianelli de Araújo J, Tessarolli V, Grizzo LT, Pessan JP, Machado MA, Buzalaf MA. Factors influencing fluoride ingestion by children. Community Dent Oral Epidemiol. 2011; 39:426-32.

[17] McDonnell ST, O'Mullane D, Cronin M, MacCormac C, Kirk J. Relevant factors when considering fingernail clippings as a fluoride biomarker. Community Dent Health 2004; 21:19-24

importante verificar se a utilização de DCRFs causaria algum mudança nos níveis de fluoreto absorvido utilizando biomarcadores. Os níveis de fluoreto nas unhas são uma das opções de biomarcadores mais estudadas para exposição de fluoreto, pois é um método não invasivo e de fácil coleta [18]. Buzalaf et al.[19] avaliaram os níveis de fluoreto em unhas quando foram utilizados dentifrícios 1100 ppm F e DCRFs. Foi observado menor concentração de fluoreto nas unhas com o DCRF do que com o dentifrício convencional. Buzalaf et al. [20] também observaram moderada correlação do fluoreto total ingerido e o fluoreto nas unhas. Este fato sugere que as unhas podem ser usadas como biomarcadores para o monitoramento da ingestão de fluoretos a partir de dentifrícios com diferentes concentrações de fluoreto.

Entretanto, de Almeida et al. [21] observaram que pequenas variações de fluoreto ingerido diariamente não podem ser detectadas nas unhas das mãos. Somando-se a isto, Lima-Arsati et al.[22] verificaram que a concentração de fluoreto nas unhas, após o uso interrompido de dentifrícios, mostra uma grande variação e não apresentou tendência à redução da concentração de fluoreto, sugerindo que a unha não é um biomarcador confiável. Um recente estudo [23] avaliou o uso da determinação de fluoreto nas unhas das mãos em crianças entre 2-7 anos de idade como preditor de risco para o desenvolvimento da fluorose dentária para a dentição permanente. Foi observado que a concentração de fluoreto nas unhas seria útil na

[18] Pessan JP, Buzalaf MAR. Historical and recent biological markers of exposure to fluoride. Buzalaf MAR (ed): Fluoride and the oral environmente. Monogr Oral Sci. Basel. Karger, 2011; 22:52-65.

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[21] De Almeida BS, Cardoso VS, Buzalaf, MAR: Fluoride ingestion from toothpaste and diet in 1-to 3-year- old Brazilian children. *Community Dent Oral Epidemiol* 2007; **35**: 53–63.

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[23] Buzalaf MAR, Massaro CS, Rodrigues MHC, Fukushima R, Pessan JP, Whitford GM, Sampaio FC. Validation of fingernail fluoride concentration as predictor of risk for dental fluorosis. Caries Res 2012; 46:394-400.

pesquisa de saúde pública, uma vez que tem potencial para identificar cerca de 80% das crianças em risco de desenvolver fluorose dentária.

Diante dos dados relatados acima [24] não existem evidências clínicas da efetividade de DCRFs suplementados com estes sais de polifosfatos quanto a progressão de lesões de cárie em comparação a formulações convencionais e [25] não existem estudos longitudinais avaliando as unhas como biomarcadores de exposição ao fluoreto a partir de DCRFs em crianças pequenas. Desse modo, o objetivo principal do presente estudo foi avaliar a eficácia clínica anticárie do uso de DCRFs suplementado com polifosfatos e se as unhas seriam um bom biomarcador ao longo do tempo quando infantes fizessem uso de dentifrícios DCRF/polifosfato e dentifrícios com 1100 ppm F.

Assim este estudo foi dividido em dois capítulos:

Capítulo 1: Efeito de dentifrícios com reduzida concentração de fluoreto suplementados com Cálcio e Fosfato na cárie dentária.

Capítulo 2 – Níveis de Flúor nas unhas de crianças de 18-30 meses de idade após o uso de dentifrícios com 500 ppm/ F suplementados e 1100 ppm/F.

[24] Wong MC, Clarkson J, Glenny AM, Lo EC, Marinho VC, Tsang BW, Walsh T, Worthington HV. Cochrane reviews on the benefits/risks of fluoride toothpastes. J Dent Res 2011; 90:573-9.

[25] Osuji OO, Leake JL, Chipman ML, Nikiforuk G, Locker D, Levine N. Risk factors for dental fluorosis in a fluoridated community. J Dent Res 1988; 12:1488-92.

Capítulo 1

Efeito de dentifrícios com reduzida concentração de fluoreto suplementados com polifosfatos no incremento da cárie dentária.

Efeito de dentifrícios com reduzida concentração de fluoreto suplementados com polifosfatos no incremento da cárie dentária.

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Resumo: Dentifrícios com concentração reduzida de fluoreto (DCRF) têm sido recomendados para crianças com o objetivo de se minimizar a ingestão de fluoreto (F) durante a escovação. Uma vez que a eficácia destas formulações ainda não foi estabelecida, alternativas como a adição de polifosfatos têm sido estudadas.

Objetivo: Este estudo clínico randomizado duplo-cego avaliou a eficácia anticárie de DCRFs suplementados com glicerofosfato de cálcio (GPCa) ou trimetafosfato de sódio (TMP) na dentição decídua.

Métodos: Crianças com idade média de 48 meses foram divididas em três grupos de acordo com o dentifrício utilizado: 500 ppm F com 1% de TMP (“500 TMP”, n=206), 500 ppm F com 0,25% de GPCa (“500 GPCa”, n=193) e 1100 ppm F (n=201). Exames clínicos foram realizados (ceo-s) no início e aos 18 meses do estudo, e o incremento de lesões de cárie (IC) foi calculado. Os dentifrícios e a experiência prévia de cárie (EPC) foram considerados como fatores de variação e os dados submetidos a ANOVA (2 critérios) e ao teste Student-Newman-Keuls ($p<0,05$).

Resultados: O IC foi significativamente menor com o uso do dentifrício 500 TMP ($p<0,05$). Os dentifrícios 1100 ppm F e 500 GPCa apresentaram IC similar ($p=0,275$). Não houve diferenças significativas entre os dentifrícios nas crianças sem EPC para ceo-s inicial, ceo-s final e IC. Nas crianças com EPC, o dentifrícios 500 TMP e 500

GPCa apresentaram menores valores de IC quando comparado ao 1100 ppm F ($p>0,05$).

Conclusão: O estudo sugere que os DCRFs com polifosfatos apresentam eficácia anticárie semelhante ou superior à de um dentifrício com 1100 ppm F.

Introdução

Nas últimas décadas, tem sido observado um declínio expressivo na prevalência da cárie dentária em diversas partes do mundo, o qual tem sido atribuído principalmente à fluoretação da água de abastecimento e ao uso de dentifrícios fluoretados [1]. O uso difundido dos dentifrícios, entretanto, tem sido associado a um aumento da prevalência de fluorose dentária, especialmente quando dentifrícios contendo 1000 ppm F ou mais são utilizados por crianças com idade abaixo de 5-6 anos [2].

Alternativas para se reduzir a ingestão de fluoreto e, consequentemente, minimizar o risco de desenvolvimento da fluorose dental, incluem a redução da quantidade de dentifrício colocada na escova, bem como a supervisão da criança durante a escovação quanto à quantidade de dentifrício usada e expectoração deste após a escovação. Uma vez que tais medidas dependem primariamente da adesão da criança e seus responsáveis às mesmas, existe a possibilidade do não seguimento das recomendações do profissional. Assim, tem sido proposto o uso de dentifrícios com concentração reduzida de fluoreto (DCRF) por crianças abaixo dos 6 anos de idade [3,4]. Entretanto, visto que ainda não existe consenso na literatura quanto à eficácia anticárie destes quando comparada à de dentifrícios com concentração convencional de fluoreto (1000-1100 ppm), alternativas como a redução do pH dos DCRFs [5,6,7,8,9,10] ou a suplementação destes com sais de

polifosfato[11,12,13] têm levado a resultados semelhantes ou até mesmo superiores aos de formulações convencionais.

Recentemente foi demonstrado *in vitro* que a adição de trimetafosfato de sódio (TMP) a um DCRF (500 ppm F) promoveu uma menor perda mineral do esmalte em comparação a um dentifrício convencional (1100 ppm F)[11,12] . Semelhantemente, a adição de 0,25% de glicerofosfato de sódio (GPCa) a um DCRF produziu um efeito anticárie similar ao de um dentifrício com 1100 ppm F em um estudo *in situ* [13]. Não existem, no entanto, evidências clínicas da efetividade de DCRFs suplementados com estes sais quanto a progressão de lesões de cárie em comparação a formulações convencionais. Desta forma, com base nos estudos supracitados, o objetivo do presente estudo foi avaliar a eficácia anticárie de DCRFs suplementados com GPCa ou TMP na dentição decídua. A hipótese nula testada foi a de que os DCRFs apresentariam eficácia anticárie similar à do dentifrício convencional.

Materiais e Métodos

Delineamento experimental

O presente estudo clínico randomizado foi aprovado pelo Comité de Ética em Pesquisa Humana da FOA-UNESP (Protocolo 2006-014112- Anexo A). O objetivo foi comparar a eficácia anticárie de dentifrícios contendo 500 ppm F suplementados com GPCa ou TMP à de um dentifrício contendo 1100 ppm F. A população alvo foi composta por crianças com idade entre 18 e 60 meses, matriculadas em creches de duas cidades do Estado de São Paulo, Brasil. Os objetivos do estudo foram minuciosamente explicados aos pais ou responsáveis, a partir da leitura da Carta de Esclarecimento, seguida da assinatura do Termo de Consentimento Livre e Esclarecido. As crianças foram aleatoriamente divididas em 3 grupos de acordo com

o tipo de dentifrícios, seguindo um protocolo duplo-cego. Orientações sobre higiene bucal e quantidade de dentifrício a ser colocada na escova foram transmitidas aos pais/responsáveis e o número de escovações foi estabelecido em 2 vezes ao dia. O índice de cárie (ceo-s) inicial e após 18 meses foi avaliado por dois examinadores previamente calibrados.

Áreas de estudo

O estudo foi realizado em duas cidades do interior do Estado de São Paulo, Brasil: (1) Araçatuba, latitude 21º12'32" sul e longitude 50º25'58" oeste, temperatura média de 27° C, fator IDH de 0,848 e concentração média de fluoreto na água de abastecimento de 0,7 ppm; (2) Fernandópolis, latitude 20º17'02'' sul e longitude de 50º'14'47'' oeste, temperatura média de 25,5^{o} C, fator IDH de 0,823 e concentração média de fluoreto de 0,7 ppm.

Seleção da amostra

Baseado em um estudo piloto que avaliou o índice de cárie em 50 crianças de 18 a 60 meses (0,1 ±0,48), foi estabelecido um número de 125 crianças para cada grupo nesse estudo (α= 0,05 e β=20%), prevendo uma desistência de 20% dos voluntários ao final do ensaio clínico.

Os critérios de inclusão das crianças participantes foram: (1) residir em um dos municípios supracitados; (2) apresentar idade entre 18 a 60 meses; (3) assinatura do termo de consentimento livre e esclarecido pelos pais ou responsáveis; (4) apresentar um bom estado geral de saúde; e (5) não ter participado de nenhum outro estudo clínico nos 3 meses anteriores a seleção. Os critérios de exclusão foram: (1) desistência voluntária; (2) uso de bochechos orais ou outro tipo de dentifrícios; e (3) não cumprimento do protocolo experimental. Os

sujeitos da pesquisa foram selecionados em creches de rede pública, localizadas em áreas urbanas das duas cidades supracitadas, sendo que 15 creches municipais foram incluídas no estudo, totalizando 747 crianças. Destas, 600 crianças concordaram em participar do estudo e apresentavam os critérios necessários para sua inclusão. Para garantir uma distribuição aleatória das crianças quanto aos grupos experimentais, as salas de aulas foram consideradas como unidades de tração, de forma que só um tipo de dentifrício foi distribuído aos alunos de cada sala de aula. Optou-se por este planejamento para que pudéssemos controlar o uso dos dentifrícios, assim como monitorizar as reposições dos dentifrícios pelas salas de aula, com a colaboração das professoras, bem como a escovação supervisionada diariamente nas creches. A distribuição aleatória das crianças nos diferentes grupos foi realizada por um dos pesquisadores, o qual teve-se o cuidado de se incluir números semelhantes de crianças das diversas faixas etárias, assim como, números semelhantes de meninos e meninas nos 3 grupos. Os voluntários foram divididos em: grupo 1 (G1): dentifrício com 500 TMP; grupo 2 (G2): dentifrício com 1.100; e grupo 3 (G3): dentifrício com 500 ppm GPCa. O fluxograma com a distribuição inicial e final dos voluntários está esquematizado na Figura 1.

Formulação dos dentifrícios

Os dentifrícios experimentais continham os seguintes componentes: dióxido de titânio, carboximetilcelulose, metil-p-hidroxibenzoato de sódio, aroma, sacarinato de sódio, óleo de menta, glicerina, sílica hidratada, lauril sulfato de sódio e água. O fluoreto foi acrescido às formulações para se obter uma concentração final de 500 µg F/g (na forma de NaF, Merck™, Germany) e adição de 1% de trimetafosfato de sódio (TMP, Sigma™ - Aldrich Co., USA) ou 0,25% de glicerofosfato de cálcio (GPCa, Sigma™ - Aldrich Co., USA, dl, 50% α- and 50% β-isomer). Como controle

positivo, foi utilizado o dentifrício 1100 ppm F, o qual continha a mesma formulação base. Os dentifrícios foram previamente submetidos a testes de composição, valor de pH, densidade, espuma, abrasividade, consistência, propriedades reológicas, ensaios toxicológicos, ensaios microbiológicos e dosagem de fluoreto, cálcio e fosfato, pela Associação Brasileira de Odontologia – ABO e BITUFO (Indústria e Comércio, Itupeva, SP, Brasil) (Anexo B).

Protocolo para o uso de dentifrício

As diretoras e as professoras das creches e pais foram instruídas sobre o protocolo do estudo clínico. Os pais/responsáveis foram encarregados da escovação dos dentes das crianças, tendo sido instruídos a fazê-lo 2 vezes ao dia (manhã e noite), durante 1 minuto, utilizando uma quantidade de dentifrício na escova correspondente ao tamanho de um “grão de arroz”. Para a padronização da escovação, os pais foram previamente treinados e orientados durante uma palestra. Também foram orientados a não realizar a escovação fora do período determinado. Kits para higienização bucal contendo 1 escova dental infantil e 4 tubos de dentifrícios (50 g cada), além de um folheto sobre os cuidados de higiene bucal e instruções aos pais, foram fornecidos a cada criança participante, sendo redistribuídos a cada 3 meses, e dispostos na unidade escolar devidamente identificados, quando necessário sua reposição. Para assegurar o cumprimento do protocolo do estudo, foi permitido que outros membros da família utilizassem os dentfrícios fornecidos.

As crianças foram examinadas a cada 6 meses, nas respectivas creches onde se fez a inspeção bucal, por dois cirurgiões-dentistas. A cada exame, os avaliadores realizaram a limpeza com escova ou gaze, sendo os dentes secos com gaze para melhor visualização das superfícies dentárias. O exame clínico foi realizado somente

por inspeção visual com auxílio de espelho bucal, e a anotação em formulário realizada através de uma abordagem sistemática por quadrante para o exame de cárie dentária por superfície (Anexo C).

O índice ceo-s (superfícies cariadas, com extração indicada ou obturadas) foi utilizado neste estudo[14]. Para molares, foram consideradas cinco superfícies (oclusal, mesial, distal, vestibular e lingual) e para os incisivos e caninos, quatro superfícies (mesial, distal, vestibular e lingual). No caso de diagnóstico de extração indicada (e), todas as faces foram anotadas nesta condição. Para a confirmação do diagnóstico de lesão de cárie, uma sonda clínica com ponta inativa foi utilizada. Na dúvida quanto ao diagnóstico final, a face dentária foi codificada como sadia. Foram seguidos os critérios e os códigos estabelecidos pela Organização Mundial de Saúde (OMS, 1997). Para o processo de randomização consideramos os seguintes fatores: (1) Índice inicial de cárie; (2) Idade dos sujeitos da pesquisa; (3) Gênero dos sujeitos da pesquisa. Após a coleta dos dados obtidos no exame de ceos inicial, as crianças foram classificadas quanto a experiência prévia de cárie, para somente então distribuí-las entre os grupos do estudo, assegurando-se, desta forma, uma maior homogeneidade entre estes. A concordância intra e inter-examinador foi determinada usando o teste kappa aplicado aos exames clinicos inicial e após 7 dias realizados em 30% dos voluntários. O valor de κ para concondância intra-examinadores foi de 0,85 e a confiabilidade inter-examinadores apresentou um κ de 0,88.

Os dados de ceo-s inicial e após 18 meses, bem como da progressão/incremento da cárie dentária ao final do ensaio clínico (ceo-s após 18 meses menos ceo-s inicial), foram codificados, tabulados e armazenados em um banco de dados (Microsoft Office Excel® 97-2003). Para as variáveis dependentes consideramos os valores do ceos inicial, ceos final e incremento de cárie dentária.

Foram considerados como fatores de variação os dentifrícios e a experiência prévia de cárie. Os dados foram submetidos a análise de variância (2 critérios) seguidos pelo teste de Student-Newman-Keuls. A análise estatística foi realizada o software SigmaPlot versão 12,0 e o limite de significância adotado foi de 5%.

Resultados

A idade média (± DP) de acordo com os dentifrícios empregados para higiene bucal das crianças foi 48 meses (± 12). Cada grupo incluiu aproximadamente mesmo número de indivíduos de ambos os gêneros e médias etárias semelhantes (Tabela 1). A taxa de perda de voluntários durante os 18 meses do estudo foi de 17%, 37% e 18%, respectivamente para as crianças que utilizaram os dentifrícios 500 TMP, 500 CaGP e 1100 ppm F.

Não houve diferenças significativas entre os grupos do estudo quanto ao ceo-s inicial (Tabela 1). O ceo-s final aumentou em relação ao valor inicial, não havendo diferenças significativas entre os grupos (*n*=456). Quanto ao incremento de cárie, entretanto, as crianças que utilizaram o dentifrício 500 TMP mostraram um incremento de cárie significantemente menor quando comparado ao dentifrício 1100 ppm F, não havendo diferenças significativas entre o grupo 500 GPCa e os demais (Tabela 1).

No início do ensaio clínico, os voluntários com experiência prévia de cárie representavam 31,1%, 29,5% e 30,8%, respectivamente para os grupos 500 TMP, 500 GPCa e 1100 ppm F (Tabela 2). Ao final do estudo, esta porcentagem se manteve para os grupos 500 TMP (32,2%) e 1.100 ppm F (30,5%). Para o grupo 500 GPCa ocorreu um aumento (39,7%).

Considerando as crianças sem experiência de cárie, não houve diferença estatística entre o ceo-s inicial e após 18 meses das crianças alocadas nos

diferentes dentifrícios. Observou-se um incremento de cárie para as crianças sem experiência de cárie, porém sem diferenças significativas entre os grupos. Para as crianças com experiência de cárie, o incremento de cárie foi maior quando as crianças utilizaram o dentifrício com 1100 ppm F, porém não diferiu estatisticamente do incremento nas crianças do grupo 500 GPCa. O dentifrício 500 TMP promoveu o menor incremento de cárie nas crianças com experiência de cárie.

Discussão

A efetividade de dentifrícios fluoretados tem sido alvo de um grande número de estudos clínicos e revisões de literatura nas últimas décadas, existindo forte evidência de sua efetividade na prevenção de cárie em crianças e adolescentes (fração prevenida de CPO-S de 24%) [15]. De acordo com uma metanálise recente, entretanto, até o momento não existem evidências sobre a efetividade clínica de DCRFs (500-550 ppm F) [16], o que tem intensificado o número de trabalhos laboratoriais e clínicos na tentativa de produzir formulações com menor concentração de fluoreto [5,6,7,10,11,12,13,17,18].

O presente estudo avaliou a progressão da cárie dentária em pré-escolares com e sem experiência prévia de cárie que fizeram uso de DCRFs suplementados com TMP e GPCa. Os resultados mostraram que os DCRFs levaram a menores incrementos de ceo-s quando comparados ao dentifrício convencional, em crianças residentes em área com água de abastecimento fluoretada. Estudos clínicos prévios mostraram que dentifrício sem fluoreto suplementado com 3% de TMP reduz a progressão de cárie quando comparado a um dentifrício placebo [19], porém com fluoreto (1000-1500 ppm) não houve melhora na redução do incremento de cárie [20]. A associação entre fluoreto (1000 ppm F, MFP) e 0,13% de GPCa apresentou melhor efeito anticárie quando comparado ao dentifrício com MFP [21]. Entretando, um

estudo *in situ* [22] mostrou que um dentifrício fluoretado (1500 ppm, MFP) com 0,13% de GPCa apresentou similar capacidade de reduzir a perda mineral que o controle (1500 ppm F, MFP). Ao contrário dos estudos anteriores, o presente estudo utilizou estes polifosfatos em formulações com 500 ppm F (utilizando NaF como sal fluoretado) que foram previamente testadas *in vitro* e *in situ* para determinar a proporção entre os ativos que produziriam o melhor efeito anticárie.

Em resumo, o efeito cariostático dos sais de polifosfatos orgânicos e inorgânicos está relacionado com sua habilidade de interagir com o esmalte dentário [23,24,25]. O TMP é um polifosfato inorgânico capaz de se ligar aos sítios de fosfato na superfície do esmalte [23,26] e permanecer por mais tempo adsorvido do que outros polifosfatos[23]. Esta interação forma uma camada protetora na superfície do esmalte, a qual impede a difusão ácida e facilita a difusão de cálcio [12,26] bem como de fluoreto [11] para o interior do esmalte. Quando o TMP é associado ao fluoreto, existe um efeito sinérgico que depende da proporção destes sais no produto [11,12,27]. Associações TMP/F com proporção molar entre 1,2:1 e 3,7:1 tendem a produzir menores perdas minerais. Estudos *in vitro* [11] e *in situ* (dados não publicados) confirmam esta observação, visto que a associação 500 ppm F a 1% ou 3% de TMP produziu resultados superiores ao dentifrício 1100 ppm F. Estes estudos também demonstraram que a concentração de fluoreto e cálcio no esmalte e no biofilme foram similares aos dentifrícios contendo 1100 ppm F.

O GPCa é um polifosfato orgânico e, como relatado para o TMP, também apresenta capacidade de adsorção à superfície do esmalte através dos radicais de fosfato da molécula [23,24]. Entretanto, o GPCa apresenta ação em diferentes níveis do processo de desmineralização [28.] Quando o fosfato orgânico é adsorvido à superfície do esmalte, praticamente todos os grupos fosfato são neutralizados pelo cálcio e hidroxila da hidroxiapatita [24]. O cálcio do GPCa é liberado e disponibilizado próximo

ao esmalte. Da mesma forma que para o TMP, uma adequada proporção molar entre GPCa e fluoreto deve ser obtida, visto que diferentes proporções entre GPCa/F resultam em diferentes reações com o esmalte *in vitro* (dados não publicados). A porcentagem mais adequada de GPCa quando associado ao dentifrício 500 ppm F foi 0,25% (proporção molar 2,2:1), razão pela qual esta concentração foi utilizada no presente estudo. Estes resultados foram confirmados em estudo *in situ*, demonstrando que, além do efeito anticárie produzido pela interação com o esmalte, a disponibilidade de cálcio e fosfato a partir do GPCa no biofilme dentário levaram a um efeito anticárie similar ao dentifrício 1100 ppm F [13]. Cabe ressaltar que a concentração de fluoreto, tanto no biofilme quanto no esmalte, foi similar à obtida com o uso de um dentifrício com 500 ppm F sem GPCa, indicando que o fluoreto esteve disponível no meio bucal. Este aspecto é de alta relevância, dada a preocupação quanto à manutenção da solubilidade do fluoreto no produto quando uma fonte de cálcio é adicionada [4].

A adição de polifosfatos em dentifrícios fluoretados com 500 ppm F aumentou signficativamente o efeito cariostático destes em crianças com experiência prévia de cárie. Neste grupo de crianças foi possível observar um menor incremento de cárie com os dentifrícios suplementados com polifosfatos quando comparado ao dentifrício 1100 ppm F. Entretanto, em crianças sem experiência prévia de cárie observou-se ausência de diferença entre os dentifrícios testados. Efeito semelhante foi observado no estudo de Lima et al. [29] ao comparar o desempenho clínico de um DCRF (sem adição de polifosfatos) a um DC, em crianças residentes em uma área sem água fluoretada. Estes dados, em conjunto, reforçam o conceito de que para esta parcela da população, o uso de DCRF pode ser uma opção viável, uma vez que leva em conta tanto os riscos como os benefícios destes quanto ao controle da cárie e à ingestão de F durante a escovação [2,4,30].

De acordo com os critérios da Associação Dental Americana (ADA) [31], um dentifrício demonstra superioridade quando (1) é possível observar diferença significante ($p<0,05$) entre o produto testado e o dentifrício controle e (2) se esta resposta não for maior que 90% do valor médio obtido para o dentifrício controle. No presente estudo, o dentifrício 500 TMP atendeu a ambos os critérios sendo, portanto, classificado como *superior* ao controle positivo (DC). O guia para protocolos clínicos da ADA recomenda, nesta situação, que a força da análise seja no mínimo de 80% [32]. O poder do teste realizado no presente estudo, considerando a interação entre os fatores de variação (dentifrício e experiência prévia de cárie) foi de 84%. O dentifrício 500 GPCa, entretanto, somente atendeu ao segundo critério da ADA sem, no entanto, apresentar diferença significativa quando comparado ao DC. Desta forma, segundo esta classificação, o dentifrício 500 GPCa deve ser considerado como *equivalente* ao dentifrício controle. Cabe ressaltar, entretanto, que o incremento de ceo-s no grupo DC foi praticamente o dobro do observado para o grupo 500 GPCa. Desta forma, acredita-se que a ausência de diferença significativa se deva à grande perda de voluntários ocorrida no grupo 500 GPCa, principalmente no subgrupo de crianças sem experiência de cárie.

Ressalta-se aqui que a suplementação com polifosfatos é apenas uma das opções para se aumentar a efetividade de dentifrícios fluoretados no controle da cárie dentária. Uma alternativa que também tem apresentado resultados promissores é a redução do pH do dentifrício, de forma a aumentar sua reatividade com o esmalte dental [7], além de aumentar as concentrações de F no esmalte [5], no biofilme [33] e na saliva [34] em comparação a um DC em pH neutro. Uma formulação de DCRF acidulada foi testada em um estudo clínico randomizado recente em crianças com experiência prévia de cárie, tendo sido demonstrado que o incremento de cárie nas crianças utilizando esta formulação não foi significativamente diferente

do observado para os grupos utilizando DCs neutros e acidulados [10]. De acordo com o guia da ADA seria considerado equivalente ao dentifrício com 1100 ppm F. Entretanto, há necessidade de outros estudos para a comprovação.

Em resumo, este estudo apresenta dados concretos sobre a eficácia de DCRFs em comparação a um DC, sendo possível, portanto, rejeitar a hipótese nula do estudo. Para se atestar a superioridade ou equivalência de um novo dentifrício segundo as normas da ADA, entretanto, são necessários pelo menos dois estudos clínicos independentes. Desta forma, estudos futuros ainda são necessários, idealmente utilizando métodos de diagnóstico que considerem estágios não cavitados das lesões de cárie, como o critério Nyvad ou ICDAS, com o objetivo de verificar o efeito dos produtos não somente na progressão da doença, mas também na regressão das lesões não cavitadas.

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Relevância do estudo na prática clínica

- O desenvolvimento de dentifrício com concentração reduzida de flúor é de extrema importância devido às várias condições que as crianças de baixa idade estão expostas.
- Com os resultados da presente proposta é que poderemos disponibilizar um dentifrício com concentração reduzida de flúor (500 ppm) suplementado com polifosfatos que promova o efeito cariostático semelhante ao comercial

de 1100 ppm F e que venha também reduzir o risco de fluorose em crianças de baixa idade.

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Figura 1- **-** Fluxograma do delineamento experimental. Distribuição aleatória dos voluntários de acordo com os dentifrícios G1-3 utilizados durante o estudo.

Tabela 1. Médias do índice de ceo-s inicial e após 18 meses e média de incremento de acordo com os dentifrícios fluoretado.

Tabela 2. Índice médio de ceo-s inicial e após 18 meses de tratamento e incremento médio de ceo-s, de acordo com a experiência de cárie

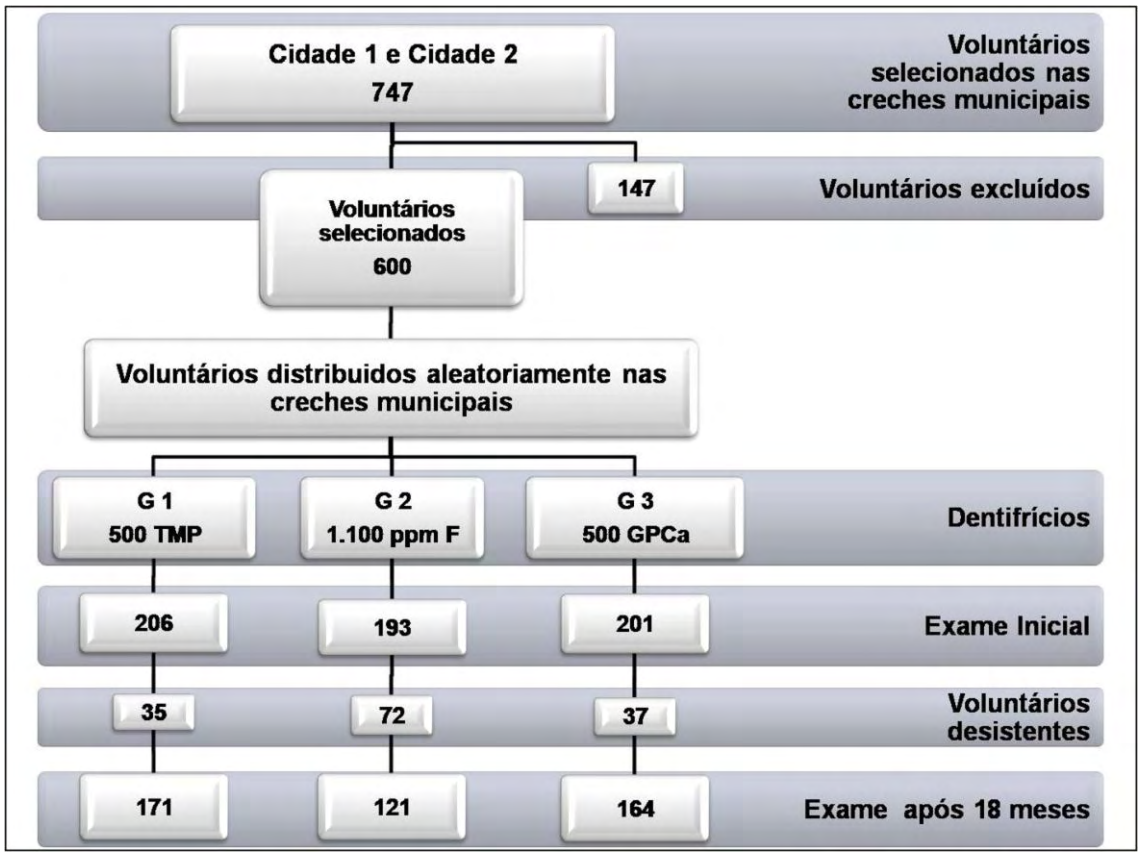

Figura 1- Fluxograma do delineamento experimental. Distribuição aleatória dos voluntários de acordo com os dentifrícios G1-3 utilizados durante o estudo.

Tabela 1. Médias do índice de ceo-s inicial e após 18 meses e média de incremento de acordo com os dentifrícios fluoretados

	Inicial			Após 18 meses		
Dentifrícios	sexo M/F	n	ceo-s (DP)	n	ceo-s (DP)	Incremento (DP)
500 TMP	102/104	206	1,88 (4,16)	171	2,08 (3,92)	0,20[a] (1,83)
500 GPCa	94/99	193	2,02 (4,31)	121	2,61 (3,41)	0,58[a,b] (1,54)
1100 ppm F	100/101	201	1,77 (4,96)	164	2,65 (4,48)	0,88[b] (2,05)
valor de p			p = 0,860		p = 0,457	p = 0,002

Letras minúsculas distintas mostram diferença entre os dentifrícios (Student-Newman-Keuls, p < 0,05). 500 TMP: dentifrício com 500 ppm F suplementado com 1% de trimetafosfato de sódio. 500 GPCa: dentifrício com 500 ppm F suplementado com 0,25% de glicerofosfato de cálcio. 1100 ppm F: dentifrício com 1100 ppm F.

Tabela 2. Índice médio de ceo-s inicial e após 18 meses de tratamento e incremento médio de ceo-s, de acordo com a experiência de cárie

		Inicial			Após 18 meses				
Dentifrícios	Cárie	n	ceo-s (DP)	valor de p*	n	ceo-s (DP)	valor de p	Incremento (DP)	valor de p
500 TMP	não	142	0,00 (4,05)	p < 0,001	116	0,03 (3,66)	p < 0,001	0,03 (1,72)	p = 0,159
	sim	64	3,77 (3,92)		55	4,14 (3,63)		0,38 (1,71)	
500 GPCa	não	136	0,00 (3,97) Ω	p < 0,001	73	0,31 (2,90) Ω	p < 0,001	0,31 (1,37) ψ	p = 0,056
	sim	57	4,04 (3,85)		48	4,90 (3,53)		0,86 (1,66) ε	
1100 ppm F	não	139	0,00 (4,95) §	p < 0,001	114	0,05 (4,48) §	p < 0,001	0,05 (2,14) λ	p < 0,001
	sim	62	3,54 (4,33)		50	5,25 (3,89)		1,71 (1,84)	

Comparação entre os valores de ceo-s inicial sem e com experiência de cárie, respectivamente: (Ω) p = 1,00 e (§) p = 0,681; após 18 meses de tratamento: (Ω) p = 0,638 e (§) p = 0,373; para o incremento de cárie: (Ω) p = 0,313 e (ψ) p < 0,079, (λ) p = 0,014, (ε) p < 0,001.
(*) Comparação entre os valores de ceo-s sem e com experiência de cárie em cada dentifrício.

Capítulo 2

Níveis de Flúor nas unhas de crianças de 18-30 meses de idade após o uso de dentifrícios com 500 ppm F suplementados e 1100 ppm F.

Fluoride levels in nails of 18-30 months old children after use of dentifrices with 500 and 1100 μg F/g.

Fluoride levels in nails of 18-30 months old children after use of dentifrices with 500 and 1100 µg F/g.

Artigo enviado à Revista Community Dentistry and Oral Epidemiology.
(Normas em Anexo)

Abstract

Objectives: The aim of this study was to evaluate F concentrations in fingernails and toenails of children aged between 18 and 30 months after use of F dentifrices supplemented with calcium glycerophosphate (CaGP) or sodium trimetaphosphate (TMP).

Methods: Children (n=56) were randomly assigned into three groups according to the dentifrice to be used: 500 µg F/g with 1% TMP, 500 µg F/g with 0.25% CaGP and 1100 µg F/g. Fingernails and toenails were monthly collected during 12 months, from the beginning of dentifrice use. F intake from diet, water and dentifrice was also determined. F was determined with the electrode after hexamethyldisiloxane-facilitaded diffusion or by the direct method, according to the samples. Data passed normality and homoscedasticity tests and were analyzed by ANOVA, followed by Student-Newman-Keuls's test ($p<0.05$).

Results: F levels in both fingernails and toenails, as well as F intake from dentifrice were similar for the groups treated with 500 µg F/g 1% TMP and 500 µg F/g 0.25% CaGP dentifrices, but significantly lower when compared with the 1100 µg F/g group ($p<0.05$). No significant differences were seen among the groups regarding F intake from the diet and water ($p>0.05$).

Conclusion: The results in the present study suggest that the nails can be used as biomarkers for fluoride exposure from dentifrices at different concentrations.

Introduction

The effectiveness of fluoride dentifrices in reducing the incidence and prevalence of dental caries has been extensively documented worldwide. However, a simultaneous increase in the prevalence of dental fluorosis prevalence has been verified (1). Several studies suggested a relationship between the early use of fluoride dentifrices and dental fluorosis development in children (2,3). This can be due to the fact that young children usually ingest high amounts of dentifrice during the toothbrushing (4,5,6). Considering that only the absorbed fluoride has an impact on the dental fluorosis development (7), the determination of fluoride absorbed levels through biomarkers has a great importance to control the risk factors for dental fluorosis.

Fluoride levels in nails are one of the most studied biomarkers for fluoride exposure, as it is a noninvasive method and easy to collect (8). Buzalaf et al. (9) evaluated the fluoride levels in nails when low-fluoride and 1,100 µg F/g dentifrices were used. They observed lower fluoride concentrations in nails when the low-fluoride concentration dentifrice was used when compared to the conventional dentifrice. Buzalaf et al. (10) also observed a moderate correlation of total fluoride intake related the nails . This fact suggest that nails can be used as biomarkers for monitoring the fluoride intake of dentifrices at different concentrations. However, de Almeida et al. (5) observed that small variations in daily fluoride intake cannot be detected in fingernails, and Lima-Arsati et al. (11) observed that the nails fluoride concentration after the interruption of the use of dentifrice, spanned a great variation e did not present any trend to reduction of fluoride concentration, suggesting that nails can not be a reliable biomarker. A recent study (12) evaluated the use of fingernail fluoride concentrations at ages 2-7 years as predictors of the risk for developing dental fluorosis in the permanent dentition, and it was observed that

fingernail fluoride concentrations should be useful in public health research, since it has potential to identify around 80% of children at risk of developing dental fluorosis.

Regarding fluoride concentration in fingernails and toenails, contradictory results are also found in the literature. Corrêa-Rodrigues et al. (13) assessed fingernails and toenails as biomarkers of subchronic exposure to fluoride when fluoride dentifrice was used (1,570 µg F/g). They found no significant differences between fingernails and toenails and suggested that the peak F concentration observed in nails can be a suitable biomarker in young children. In some studies, fingernails were shown to have higher fluoride concentration than toenails, what can be attributed to a lower exposure of toenails to external contamination (9,14,15).

In addition, there are no longitudinal studies evaluating nails as biomarkers of exposure to low-fluoride dentifrices in young children. Thus, it is important assess nails (fingernails and toenails) as biomarkers of fluoride intake when a low fluoride dentifrice is used, since nowadays there is a trend to indicate dentifrice with low-fluoride concentration, and low-fluoride dentifrices with some supplementation have showed positive results both in high and low caries activity (16,17 and unpublished data).

Material and Methods

Experimental design

The protocol of the present study was revised and approved by the local Human Research Ethics Committee (Protocol #2006-01412), and the legal responsible for the children participating in the study read and signed a informed consent. For this study, children (n=56) from a preventive program conducted at the Baby Clinic from Araçatuba Dental School (18) were selected. The sample size was based on a pilot study, to ensure α e β errors of 5% and 10%, respectively. Children

were aged between 18 and 30 months and were not using fluoride dentifrices prior to the beginning of the study. They were randomly divided into 3 groups, according to the type of dentifrice: 500 µg F/g supplemented 1% sodium trimetaphosphate (TMP); 500 µg F/g supplemented with 0.25% calcium glycerophosphate (GPCa) and 1,100 µg F/g. The dentifrices and toothbrushes were freely provided for use to the children twice/daily and the responsible were instructed to apply a small amount of the dentifrice (around 0.15 g). The nail samples were collected monthly, in separate labeled vials for the fingernails and toenails for later analysis of fluoride concentration. In order to estimate the total fluoride intake of the children, fluoride intake from diet was monitored by the duplicate plate method (19), and fluoride ingested from dentifrice was determined by subtracting the amount of fluoride recovered after brushing from the amount originally placed on the children's toothbrush. Fluoride was determined in the samples after overnight hexamethyldisiloxane-facilitated diffusion (20,21) or by the direct method, according to samples.

Nail Sampling

The nails of the volunteers were cut once a month by the caregivers The first samples were collected one day before starting the dentifrice use. Children were not allowed to use nail varnish throughout the experiment, as it may contain F (22). Fingernails and toenails from the all digits were cut and pooled in labeled vials, separately for fingernails and toenails, totaling 24 samples (12 from fingernails and 12 from toenails) for each child. Nail clippings were cleaned with deionized water using an interdental brush, sonicated in deionized water for 10 min, dried at 37° C and weighed prior to fluoride analysis. Whenever the weight of the pooled samples was higher than 20 mg, analysis were conducted in duplicate.

Estimation of fluoride intake from dentifrice

Attempts were made to simulate real-life conditions. The child's responsible was oriented to bring the child's toothbrush and the dentifrice that was being used at home. The toothbrush was weighed and then the responsible spread dentifrice onto the toothbrush, according to normal practice, and the final weight of the toothbrush plus dentifrice was recorded. This provided information on the amount of fluoride loaded onto the brush. Brushing was performed by the child with assistance of responsible and under the observation of the examiner. The expectorated material was collected in a plastic vial and the volume recorded. The toothbrush was thoroughly rinsed in 30 ml of deionized water and it was collected in the same vial containing the expectorate. The amount of F left on the toothbrush and the amount expelled were added, to give the total amount of fluoride not ingested. The fluoride concentration in the toothbrush was obtained by multiplying the weight of dentifrice applied on the toothbrush and the dentifrice concentration. Following, the amount of fluoride ingested was indirectly derived by subtracting the amount initially loaded onto the toothbrush from the amount of fluoride expelled and left on the toothbrush. The determination of daily fluoride intake from the dentifrice considered brushing frequency (twice per day). Children were also weighed in order that their fluoride intake could be expressed in µg/kg body weight/day (4).

Estimation of fluoride intake from water an diet

Water samples were collected from the volunteers' houses monthly, on the same days when nail clippings were obtained. The water samples were collected in plastic vials, and frozen (-20° C) until analysis. For the estimation of fluoride intake

from the diet, duplicate portions of all foods and drinks consumed by each child in 24 h were collected, at three separate days, with an interval of 6 months between each collection. The constituents of the diet were collected in plastic vials. Parents were requested to remove parts of foods not normally eaten, such as seeds, skin, and bones, before placing the food in the vials (13). Samples were homogenized in a blender using deionized water (when necessary), and the total weight was measured. An aliquot sample of 50 ml of the homogenized foods was frozen at -20° C until analysis.

Fluoride analysis

Fluoride analysis of water samples was done by the direct method using an ion-specific electrode (Orion 9409-BN, Orion Research, Inc. Beverly, MA, USA), after sample buffering with an equal volume of total ionic strength adjustment buffer (TISAB II). Diet and fingernail samples were analyzed for fluoride after overnight hexamethyldisiloxane (HMDS)-facilitated diffusion (20,21), using the same electrode and a miniature calomel reference electrode (Accumet, no. 13-620-79: Fischer Scientific, Pittsburgh, PN, USA), coupled to a potentiometer (Orion 720 A^{plus}, Orion Research, Inc. Beverly, MA, USA). Analyses of F content from diet and water were conducted in duplicate. F concentration in each sample of diet was multiplied by the total weight of the diet collected in each day. For fluoride intake from dentifrice, the collected solution (toothbrush residue + child's expectorate) was homogenized and an amount of 0.25 mL of 1M HCl was added to 0.25 mL of the collected solution. Then, this mixture was incubated for 1 hour at 45°C, under agitation. Following, the mixture was neutralized with 0.5 mL of 1M NaOH and buffered with 1 mL of TISAB II. The samples were analyzed in duplicate, using the specific ion electrode (Orion 9409-BN, Orion Research, Inc. Beverly, MA, USA) coupled to potentiometer. Fluoride

standards ranged from 0.25 to 8 µg F/ml. Fluoride intake from the dentifrice was calculated by subtracting the fluoride content in the toothpaste from the fluoride in solution. The daily toothbrushing frequency and weight of each child were used to calculate the ingested in terms of µg F/kg of body weight/day.

Statistical Analysis

For statistical analysis, SigmaPlot 12.0 software was used and the significance limit was set at 5%. Data passed normality and homoscedasticity tests. For fluoride concentrations in fingernails and toenails, as well as fluoride intake from diet, data were submitted to 2-way ANOVA followed by Student-Newman-Keuls's test. Data of intake from dentifrice and water were submitted to 1-way ANOVA followed by Student-Newman-Keuls's test. Pearsons's correlation was used to compare fluoride intake from dentifrice and fluoride concentration from nails.

Results

Fluoride concentrations in both fingernails and toenails (Table 1) were similar between the groups 500 µg F/g CaGP and 500 µg F/g TMP ($p = 0.474$), but significantly lower than the values found for the 1,100 µg F/g ($p < 0.001$). Overall, fluoride concentrations in fingernails were higher than in toenails ($p < 0.001$); significant differences were observed between fingernails and toenails in the groups with 1,100 µg F/g ($p = 0.001$) and the 500 µg F/g CaGP ($p = 0.006$). Figure 1 shows the mean of fluoride concentration from fingernails and toenails over time according to dentifrice. Lower fluoride concentrations are observed at the beginning of the study, with peak fluoride concentrations at 30 and 60 days after children started to use the dentifrices. The comparison of fluoride in the fingernails and toenails showed a similar trend line between the groups of children who used dentifrices with 500 µg

F/g (Figure 2). Differences between fingernails and toenails were observed only in peak areas.

The highest fluoride intake from dentifrice concentration (Table 1) was found in the 1,100 µg F/g dentifrice when compared with 500 µg F/g dentifrices ($p \leq 0.041$), which showed similar values ($p = 0.420$). The amount of dentifrice placed on the toothbrush among the groups were similar, and the means values (SD) were: 0.19 (0.06) g for the 500 µg F/g TMP, 0.15 (0.10) g for the 1,100 µg F/g e 0.19 (0.11) g for the 500 µg F/g CaGP group ($p = 0.237$). A positive correlation (Figure 2D) was found between fluoride intake from dentifrice and fluoride concentration in nails (Pearson's $r = 0.461$, $R^2 = 0.213$, $p = 0.005$).

The fluoride concentration from diet (Table 2) did not show variation over time and no differences among the groups ($p = 0.893$). Moreover, no differences were seen among the groups for fluoride concentration in water ($p = 0.258$).

Discussion

Nowadays, nails are one of the most studied biomarkers. Buzalaf et al. (12) observed that this biomarker showed potential to identify around 80% of risk to dental fluorosis development in the permanent dentition when 2-7-year-old children were evaluated. However, few studies assessed nails as biomarker after the use of low-fluoride dentifrices. Whereas there is a strong trend to use low fluoride concentration dentifrices for children in the critical period for development fluorosis; it was evaluated the nails fluoride concentration for children considered at the risk range age when 1,100 µg F/g and 500 µg F/g TMP or with 500 µg F/g CaGP dentifrices were used.

Particularly in this study, none of the children were using fluoride dentifrice previously, differently from previous reports (5,9,13). Besides, children underwent a

routine prevention of dental caries (18). By the introduction of toothpaste on hygiene of the children, it was expected a rise in concentration of fluoride in the nails, considering it as a good biomarker (13,15). Taken into account that children have a faster growth rate (7) and a shorter length of their nails, it would be expected that the mean peak fluoride concentrations would occur before 14 weeks. A peak of 15% increase was observed with 500 -µg F/g dentifrices with the first 30 days. Using the dentifrice 1,100 µg F/g a peak of 47% increase was observed at 60 days of use of the product (Figure 1). Corrêa Rodrigues et al (13), observed some peaks in the nail fluoride concentration after 100 days of the study beginning. It is interesting to note that at 30 days, the children used toothpaste with 1,100 µg F/g, showed a 16% increase in fluoride concentration in nails close to other dentifrices. Thus, it can be observed that the nails fluoride concentration was influenced by the fluoride concentration of the dentifrice.

Buzalaf et al. (9) also noted a difference in nails fluoride concentration after use 1,100 and 500 µg F/g dentifrices, and higher values were observed when 1,100 µg F/g dentifrice was used. The values observed in the nails fluoride concentration when it was used 1,100 µg F/g are similar to the values found in the literature for both, fingernails and toenails. And these ones showed a difference between them, being found higher values for fingernails (9,13) which can also be observed in this study. The higher F concentrations in fingernails have been attributed to a higher blood supply (23). Moreover, fingernails may be more prone to external contamination by F (9). In the present study and considering 12 months, mean fluoride concentrations in fingernails and toenails were similar (1.48 and 1.53, respectively) in the 500 µg F/g TMP group, which is in agreement with some studies (13,24). However, the other groups (1,100 µg F/g and 500 µg F/g CaGP) showed difference 20% higher in fingernails than those in toenails. Studies found fluoride concentrations in fingernails

to be 25% (14) and 50% (23) higher than those in toenails. The differences between fingernails and toenails were observed when analyzing the average fluoride of 12 months (Table 1).

By analyzing month to month each type of nail, there is difference only in the peak of fluoride that were found at 1,100 µg F/g and 500 µg F/g CaGP groups (Figure 2). One factor may have been the highest concentration of fluoride in toothpastes (25) and a different profile of fluoride between fingernails and toenails (Figure 2B). The trend lines of fluoride in the fingernails and toenails to the dentifrices were similar with 500 µg F/g dentifrices (Figure 2A and 2C). The difference between the nails was only possible to be observed in the dentifrice 500 µg F/g CaGP because it presented a greater distance in the amounts of fluoride over the 12 months, even with no statistical difference. In general, the trend lines showed a similar behavior of the dentifrices, with two peaks of fluoride. However, it was early in the dentifrices with 500 µg F/g (30 and 180 days) and late for the dentifrice 1,100 µg F/g (90 and 240 days). The lowest concentrations of fluoride in the nails were observed in periods of approximately three months (Figure 2) and may be related to more severe stages of growth, deposition and bone remodeling in children (22).

Fluoride source from diet and water can vary among locations and type of water consumed (mineral or tap). These values did not differ among the groups, and the water showed lower mean values concentrations than ones found in the literature (12,26). The low fluoride concentration found in diet, can be partially explained by the higher mineral water consumption (72% of the volunteers) and low concentration found in these samples. In addition, the estimated fluoride intake from the dentifrice was statistically difference between the 1,100 µg F/g and 500 µg F/g groups, and the highest values were found in the 1,100 µg F/g group. And this can related with the fluoride concentrations obtained in the nails. The presence of correlation between

these two factors (Figure 2D) and the similar amount of dentifrice placed on the brush reinforce these findings. So, it is believed that the difference observed in nails fluoride concentration can be attributed to the values found for the fluoride intake from dentifrice.

The presence of dose-response effect between fluoride intake and its concentration in nails can be related to type of attention give to the children (18) and the their low fluoride intake from diet and water (Table 2). Furthermore, the Baby Clinic routine of periodic returns (two months) allowed children attention and appliance of parents to the use of dentifrices (quantity and frequency). Analysis over time seems to be an important factor for dose-response can verify, since there can be many variations in data (Figure 1 and 2) and there is study that did not detect the effect of fluoride intake from dentifrice (11). Thus, the results in the present study suggest that, in children submitted to clinical control, it is possible to detect fluoride exposure from dentifrice using the nails as biomarkers. This was possible because the analysis over time and similar patterns of fluoride intake from the diet and water.

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Table legends

Table 1 – Mean (SD) fluoride concentrations in nails and fluoride intake from toothpastes containing 500 µg F/g TMP, 500 µg F/g CaGP and 1,100 µg F/g.

Table 2 – Mean (SD) daily fluoride intake from the diet and water by children using dentifrices containing 500 µg F/g TMP, 500 µg F/g CaGP and 1,100 µg F/g.

Figure legends

Figure 1 – Mean fluoride concentrations in fingernails and toenails over time (days) according to the dentifrices used. (*) Asterisk indicates significant difference between the 1.100 µg F/g dentifrice and 500-µg F/g dentifrices (Student-Newman-Keuls's test, $p < 0.05$). Arrow shows peak nail fluoride concentrations after the introduction of dentifrices in the children's hygiene. Bars denote the standard deviation of the average.

Figure 2 – Mean fluoride concentrations in fingernails and toenails over time (days) according to toothpastes: A) 500 µg F/g TMP, B) 1,100 µg F/g and C) 500 µg F/g CaGP. (*) Asterisk indicates significant difference between fingernails and toenails (Student-Newman-Keuls's test, $p < 0.05$). Black trend line (polynomial) for the data of fingernails. Gray trend line (polynomial) for the data of toenails. (D) Pearson's correlation between fluoride intake from dentifrice and fluoride concentration in the nails.

Table 1 – Mean (SD) fluoride concentrations in nails and fluoride intake from toothpastes containing 500 µg F/g TMP, 500 µg F/g CaGP and 1,100 µg F/g

Toothpaste	Fingernail (µg F/g)	Toenail (µg F/g)	Intake (µg F/g)
500 µg F/g TMP	^{A}2.44 (0.40)^{a} (0.8 – 8.0)*	^{A}2.19 (0.41)^{a} (0.2 – 5.9)	5.0 (2.2)^{a} (1.4 – 9.4)
500 µg F/g CaGP	^{A}2.66 (0.38)^{a} (0.3 – 6.7)	^{B}2.15 (0.39)^{a} (0.1 – 6.6)	6.6 (3.9)^{a} (1.4 – 14.2)
1,100 µg F/g	^{A}3.26 (0.60)^{b} (0.4 – 14.8)	^{B}2.64 (0.41)^{b} (0.4 – 8.7)	11.6 (8.3)^{b} (2.1 – 32.7)

Distinct superscript lowercase letters indicate statistical significance in each analysis. Distinct superscript capital letters indicate statistical significance between Finger and toe in each Toothpaste. (Student-Newman-Keuls's test, $p < 0.05$).
* Range of variation.

Table 2 – Mean (SD) daily fluoride intake from the diet and water by children using dentifrices containing 500 µg F/g TMP, 500 µg F/g CaGP and 1,100 µg F/g

Toothpaste	Diet intake (mg F/Kg of weight)			Water (mg F/L)
	First collection	150 days	330 days	
500 µg F/g TMP	0.021 (0.015)^{a} (0.004 – 0.065)*	0.020 (0.014)^{a} (0.004 – 0.061)	0.020 (0.015)^{a} (0.009 – 0.064)	0.213 (0.077)^{a} (0.009 – 0.986)
500 µg F/g CaGP	0.018 (0.014)^{a} (0.006 – 0.073)	0.022 (0.017)^{a} (0.003 – 0.074)	0.018 (0.009)^{a} (0.006 – 0.031)	0.204 (0.059)^{a} (0.009 – 0.967)
1,100 µg F/g	0.019 (0.012)^{a} (0.002 – 0.049)	0.018 (0.011)^{a} (0.003 – 0.038)	0.014 (0.005)^{a} (0.005 – 0.021)	0.247 (0.062)^{a} (0.009 – 0.938)

Distinct superscript lowercase letters indicate statistical significance in each analysis (Student-Newman-Keuls's test, $p < 0.05$). *Range of variation.

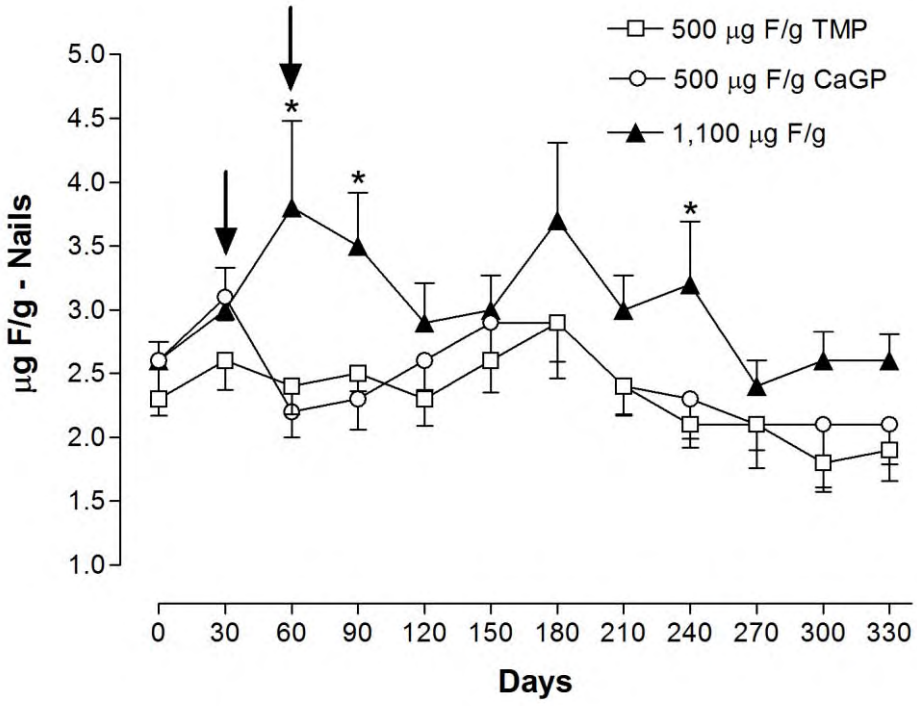


Figure 1 – Mean fluoride concentrations in fingernails and toenails over time (days) according to the dentifrices used. (*) Asterisk indicates significant difference between the 1.100 µg F/g dentifrice and 500-µg F/g dentifrices (Student-Newman-Keuls's test, $p < 0.05$). Arrow shows peak nail fluoride concentrations after the introduction of dentifrices in the children's hygiene. Bars denote the standard deviation of the average.

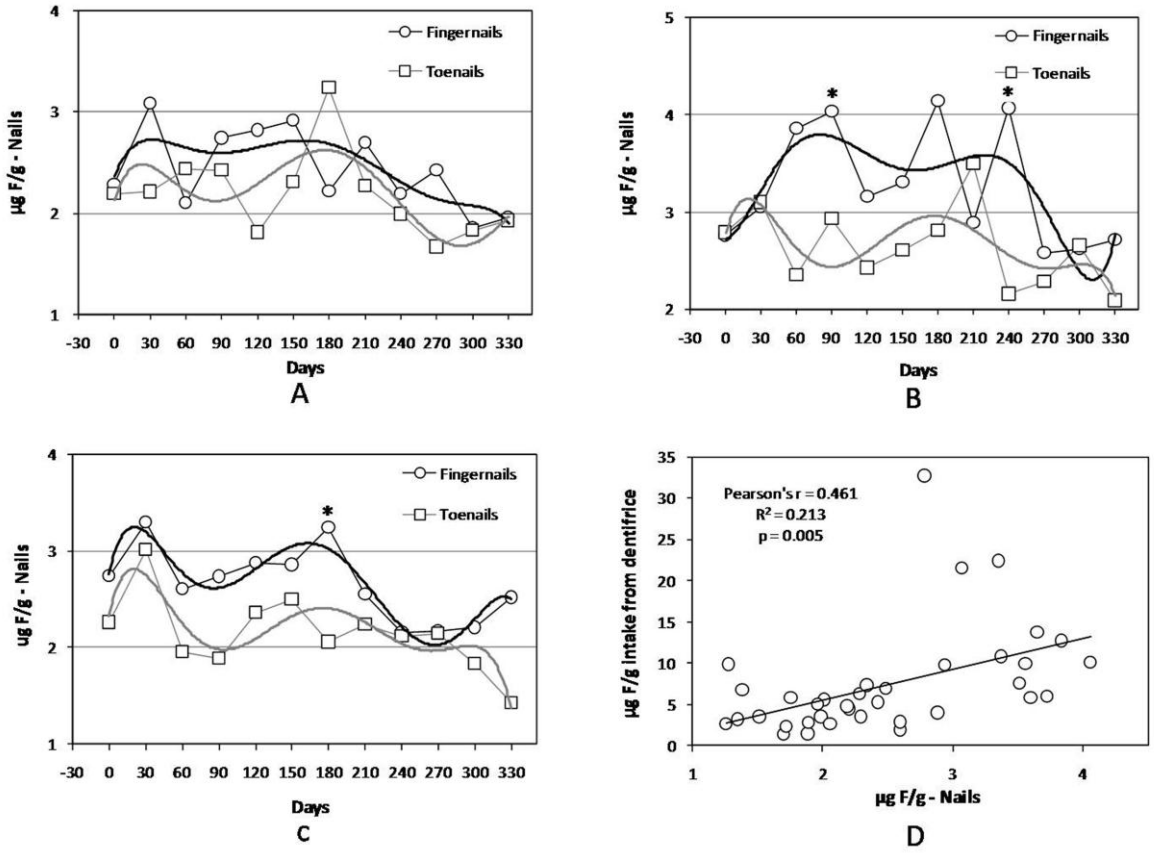


Figure 2 – Mean fluoride concentrations in fingernails and toenails over time (days) according to toothpastes: A) 500 µg F/g TMP, B) 1,100 µg F/g and C) 500 µg F/g CaGP. (*) Asterisk indicates significant difference between fingernails and toenails (Student-Newman-Keuls's test, $p < 0.05$). Black trend line (polynomial) for the data of fingernails. Gray trend line (polynomial) for the data of toenails. (D) Pearson's correlation between fluoride intake from dentifrice and fluoride concentration in the nails.

Anexos

Anexo A

unesp

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

FLS. 50
PROC. 2006-01412
RUB.

OF. 020/2008
CEP
SFCD/bri

Araçatuba, 25 de março de 2008

Referência Processo FOA 2006-01412

O Comitê de Ética em Pesquisa desta Unidade, em reunião **de 28/02/2008,** deliberou, após o parecer favorável do relator Prof. Dr. Paulo Renato Junqueira Zuim, às fls. 49, *aprovar a* ***prorrogação para 28/02/2009*** e *a* ***alteração do título*** **do projeto :"EFEITO DOS DENTIFRÍCIOS DE BAIXA CONCENTRAÇÃO DE FLÚOR SUPLEMENTADOS OU ACIDULADOS EM CRIANÇAS DE 18 a 36 MESES" para "AVALIAÇÃO LONGITUDINAL DO EFEITO DO USO DE DENTIFRÍCIOS COM BAIXA CONCENTRAÇÃO DE FLÚOR NA INFÂNCIA (EM CRIANÇAS A PARTIR DOS 18 MESES ATÉ 6 ANOS DE IDADE".**

Prof. Dr. Stefan Fiuza de Carvalho Dekon
Coordenador do CEP

Ciente.De acordo
17/03/2008
Drª.Cleide Cristina R.Martinhon

Ilma. Senhora
Drª. CLEIDE CRISTINA RODRIGUES MARTINHON
Campus de Araçatuba

Faculdade de Odontologia e Curso de Medicina Veterinária – DIRETORIA – COMITÊ DE ÉTICA EM PESQUISA
Rua José Bonifácio, 1193 CEP 16015-050 Araçatuba – SP
Tel (18) 620-3203 E-mail: diretor@foa.unesp.br

Termo de Consentimento Livre e Esclarecido

COMITÊ DE ÉTICA EM PESQUISA – CEP

(Resolução nº 01 de 13/06/98 – CNS)

TERMO DE CONSENTIMENTO ESCLARECIDO

I – DADOS DE IDENTIFICAÇÃO DO PACIENTE OU RESPONSÁVEL LEGAL

1. Nome do Paciente (Voluntário):			
Documento de Identidade nº	**Sexo:**	**Data de Nascimento:**	
Endereço:		**Cidade:**	**U.F.**
Telefone:	**CEP:**		

1. Responsável Legal:		
Documento de Identidade nº	**Sexo:**	**Data de Nascimento:**
Endereço:	**Cidade:**	**U.F.**
Natureza (grau de parentesco, tutor, curador, etc.):		

II – DADOS SOBRE A PESQUISA CIENTÍFICA

1.Título do protocolo de pesquisa: Avaliação longitudinal do efeito do uso de dentifrícios com baixa concentração de flúor na infância (em crianças a partir dos 18 meses até aos 6 anos de idade).		
Pesquisador responsável: Cleide Cristina Rodrigues Martinhon		
Cargo/função: Profa Doutora	**Inscr.Cons.Regional: 55946**	**Unidade ou Departamento do Solicitante: Departamento de Odontologia Infantil e Social**

3. Avaliação do risco da pesquisa: (probabilidade de que o indivíduo sofra algum dano como consequência imediata ou tardia do estudo).

■ SEM RISCO ☐ RISCO MÍNIMO ☐ RISCO MÉDIO ☐ RISCO MAIOR

4. Justificativa e os objetivos da pesquisa (explicitar):

Este projeto temático tem como objetivo comparar o impacto do uso clínico de pasta de dentes com concentração reduzida de flúor suplementados com cálcio e fosfato, dos 18 meses até 6 anos de idade, com uma pasta de dentes convencional, na prevalência de cárie dentária e na ingestão de flúor bem como correlacionar o índice de cárie e o possível risco de Fluorose com o tipo de dentifrício utilizado e correlacionar a concentração de flúor presente na urina e unhas com o tipo de dentifrício utilizado e o flúor ingerido pela dieta e dentifrício e também correlacionar os fatores sócio-comportamentais, biológicos e dietéticos e o processo saúde-doença cárie dentária.

5. Procedimentos que serão utilizados e propósitos, incluindo a identificação dos procedimentos que são experimentais: (explicitar)

Os pacientes serão submetidos a avaliações freqüentes quanto à incidência de cárie. A ingestão de flúor será monitorada, em parte da amostra, através da dieta duplicada e estimativa de ingestão de flúor pela pasta de dente, bem como as concentrações de flúor presente nas unhas e na urina, os dados coletados serão analisados estatisticamente, correlacionado-os ao tipo de dentifrício usado e à ingestão de flúor pela dieta e eliminação pela urina e unha.

6. Desconfortos e riscos esperados: (explicitar)

A coleta das unhas, urina e dieta não causarão desconforto ou risco às crianças.

7. Benefícios que poderão ser obtidos: (explicitar)

Através do acompanhamento longitudinal espera-se disponibilizar um dentifrício com concentração reduzida de flúor suplementado com cálcio e fosfato que promova o efeito cariostático bem como reduzir o risco de fluorose em crianças de baixa idade.

8. Procedimentos alternativos que possam ser vantajosos para o indivíduo: (explicitar)

Não há

Duração da pesquisa: 05 anos

10. Aprovação do Protocolo de pesquisa pelo comitê de ética para análise de projetos de pesquisa em / /

III - EXPLICAÇÕES DO PESQUISADOR AO PACIENTE OU SEU REPRESENTANTE LEGAL

1. Recebi esclarecimentos sobre a garantia de resposta a qualquer pergunta, a qualquer dúvida acerca dos procedimentos, riscos, benefícios e outros assuntos relacionados com a pesquisa e o tratamento do indivíduo.
2. Recebi esclarecimentos sobre a liberdade de retirar meu consentimento a qualquer momento e deixar de participar no estudo, sem que isto traga prejuízo à continuação de meu tratamento.
3. Recebi esclarecimento sobre compromisso de que minha identificação se manterá confidencial tanto quanto a informação relacionada com a minha privacidade.
4. Recebi esclarecimento sobre a disposição e o compromisso de receber informações obtidas durante o estudo, quando solicitada, ainda que possa afetar minha vontade em continuar participando da pesquisa.
5. Recebi esclarecimento sobre a disponibilidade de assistência no caso de complicações e danos decorrentes da pesquisa.
Observações complementares.

IV – CONSENTIMENTO PÓS-ESCLARECIDO

Declaro que, após ter sido convenientemente esclarecido (a) pelo pesquisador, conforme registro nos ítens 1 a 6 do inciso III, consinto em participar, na qualidade de paciente, do Projeto de Pesquisa referido no inciso II.

_____ Local, / / .

Assinatura

Testemunha

Nome:

Endereço:

Telefone:

R.G.:

Testemunha

Nome:

Endereço:

Telefone:

RG:

Anexo B

ASSOCIAÇÃO BRASILEIRA DE ODONTOLOGIA

Missão

Promover a odontologia, nacional e internacionalmente, valorizar o profissional no contexto técnico-científico e sociocultural, e contribuir com as políticas de promoção da saúde bucal da população.

Oficio DIR: Nº 0177/ Gestão 2007/2010

São Paulo, 05 de maio de 2008.

UNESP/Araçatuba
Profº Dr. Alberto Carlos Botazzo Delbem

Prezados Srs,

Estamos enviando-lhes o trabalho de análise realizado no HELP - Laboratório de Desenvolvimento e Pesquisa Ltda, sobre o produto **"DENTIFRÍCIO 500ppm Suplementado com fosfato e DENTIFRÍCIO 500ppm Suplementado com Cálcio e Fosfato"**, cuja recomendação é favorável à concessão do Selo de Qualidade.

Desta forma, autorizamos o uso do selo na embalagem do produto, obedecidas às cláusulas do contrato a ser firmado entre as partes.

Sem mais, despedimo-nos com elevados protestos de estima e apreço.

Atenciosamente,

Norberto Francisco Lubiana – CD
- Presidente -

Rua Vergueiro, 3153 - Conj. 82 - 04101-300 - São Paulo - SP - Fone/Fax: (11) 5083-4000
E-mail: abo@abo.org.br - Site: www.abo.org.br

Filiada à fdi fola

ANEXO C – Folha de Exame Clínico

EXAME CLÍNICO

Nome:
Data de nascimento:
Data do exame: _____
Presença de placa:

52 51 61 62

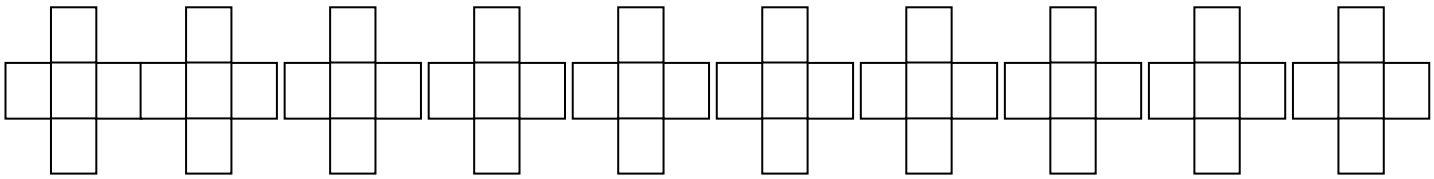
☐ ☐ ☐ ☐

Códigos:
0 - Ausência de placa evidente 1 - Presença de placa evidente

Odontodiagrama:

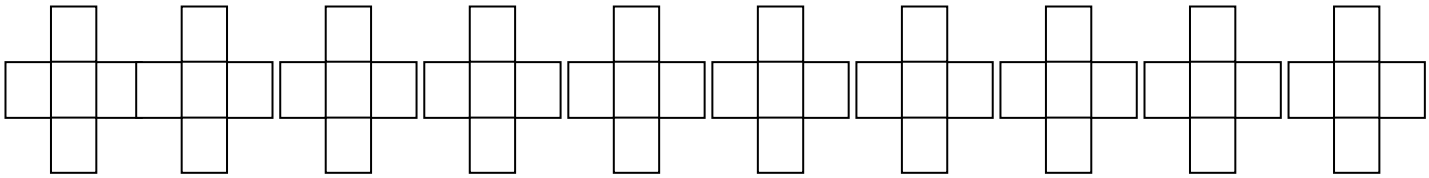
55 54 53 52 51 61 62 63 64

65



85 84 83 82 81 71 72 73 74

75



Códigos:
A dente hígido
B dente cariado
C dente restaurado e cariado
D dente restaurado sem cárie
E dente perdido por cárie
F dente permanente perdido que não seja por cárie
G selante
K Coroa não erupcionada
L Dente excluído

Comportamento durante o exame: ☐
Códigos

1- não colaborador
2- colaborador com reserva
3- colaborador

Normas : International Journal of Paediatric Dentistry

Edited By: Chris Deery
Impact Factor: 1.008
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Online ISSN: 1365-263X

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International Journal of Paediatric Dentistry publishes papers on all aspects of paediatric dentistry including: growth and development, behaviour management, prevention, restorative treatment and issue relating to medically compromised children or those with disabilities. This peer-reviewed journal features scientific articles, reviews, clinical techniques, brief clinical reports, short communications and abstracts of current paediatric dental research. Analytical studies with a scientific novelty value are preferred to descriptive studies.

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In June 2007 the Editors gave a presentation on How to write a successful paper for the *International Journal of Paediatric Dentistry.*

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Submission is considered on the conditions that papers are previously unpublished, and are not offered simultaneously elsewhere; that authors have read and approved the content, and all authors have also declared all competing interests; and that the work complies with the

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Articles for the *International Journal of Paediatric Dentistry* should be submitted electronically via an online submission site. Full instructions and support are available on the site and a user ID and password can be obtained on the first visit. Support is available by phone (+1 434 817 2040 ext. 167) or here. If you cannot submit online, please contact Reidin Vaughan in the Editorial Office by telephone (+44 (0)1865 476327) or by e-mail revaughan@wiley.com.

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Launch your web browser (supported browsers include Internet Explorer 5.5 or higher, Safari 1.2.4, or Firefox 1.0.4 or higher) and go to the journal's online submission site: http://mc.manuscriptcentral.com/ijpd

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*If you are registering as a new user.

- After clicking on 'Create Account', enter your name and e-mail information and click 'Next'.
- Your e-mail information is very important.
- Enter your institution and address information as appropriate, and then click 'Next.' - Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your area of expertise. Click 'Finish'.

*If you are already registered, but have forgotten your log in details, enter your e-mail address under 'Password Help'. The system will send you an automatic user ID and a new temporary password.

*Log-in and select 'Author Center'.

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After you have logged into your 'Author Center', submit your manuscript by clicking on the submission link under 'Author Resources'.

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* You may copy and paste directly from your manuscript and you may upload your pre-prepared covering letter. **Please note** that a separate *Title Page* must be submitted as part of the submission process as 'Title Page' and should contain the following:

• Word count (excluding tables)

• Authors' names, professional and academic qualifications, positions and places of work. They must all have actively contributed to the overall design and execution of the study/paper and should be listed in order of importance of their contribution

• Corresponding author address, and telephone and fax numbers and email address

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*You are required to upload your files.

- Click on the 'Browse' button and locate the file on your computer.
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Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rft) files (not write-protected) plus separate figure files. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS files are suitable for printing. The files will be automatically converted to HTML and a PDF document on upload and will be used for the

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Original Articles: Divided into: Summary, Introduction, Material and methods, Results, Discussion, Bullet points, Acknowledgements, References, Figure legends, Tables and Figures arranged in this order. The summary should be structured using the following subheadings: Background, Hypothesis or Aim, Design, Results, and Conclusions and should be less than 200 words. A brief description, in bullet form, should be included at the end of the paper and should describe Why this paper is important to paediatric dentists.

Review Articles: may be invited by the Editor.
Short Communications: should contain important, new, definitive information of sufficient significance to warrant publication. They should not be divided into different parts and summaries are not required.
Clinical Techniques: This type of publication is best suited to describe significant improvements in clinical practice such as introduction of new technology or practical approaches to recognised clinical challenges.
Brief Clinical Reports/Case Reports: Short papers not exceeding 800 words, including a maximum of three illustrations and five references may be accepted for publication if they serve to promote communication between clinicians and researchers. If the paper describes a genetic disorder, the OMIM unique six-digit number should be provided for online cross reference (Online Mendelian Inheritance in Man).
A paper submitted as a Brief Clinical/Case Report should include the following:

- a short **Introduction** (avoid lengthy reviews of literature);
- the **Case report** itself (a brief description of the patient/s, presenting condition, any special investigations and outcomes);
- a **Discussion** which should highlight specific aspects of the case(s), explain/interpret the main findings and provide a scientific appraisal of any previously reported work in the field.
- Please provide up to 3 bullet points for your manuscript under the heading: 1. Why this clinical report is important to paediatric dentists. Bullet points should be added to the end of your manuscript, before the references.

Letters to the Editor: Should be sent directly to the editor for consideration in the journal.

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The whole manuscript should be double-spaced, paginated, and submitted in correct English. The beginning of each paragraph should be properly marked with an indent.

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Summary should be structured using the following subheadings: Background, Hypothesis or Aim, Design, Results, and Conclusions.

Introduction should be brief and end with a statement of the aim of the study or hypotheses tested. Describe and cite only the most relevant earlier studies. Avoid presentation of an extensive review of the field.

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4. Al-Hosani E, Rugg-Gunn A. Combination of low parental educational attainment and high parental income related to high caries experience in preschool children in Abu Dhabi. Community Dent Oral Epidemiol 1998; 26: 31-36.
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Esquematização da coleta da dieta duplicada para análise da concentração de F presente na dieta dos voluntários do estudo.

Coleta do consumo diário de alimentos e bebidas

Armazenados em potes plásticos, mantendo refrigerado até a coleta

Homegeinização do conteúdo coletado

Armazenamento de 50 ml em freezer -20° para posterior análise de F

As concentrações de F das amostras da dieta foram analisadas após adição de hexametildisiloxano durante a noite (HMDS) difusão facilitada, utilizando um eletrodo flúor-sensível e um de referência de calomelano,

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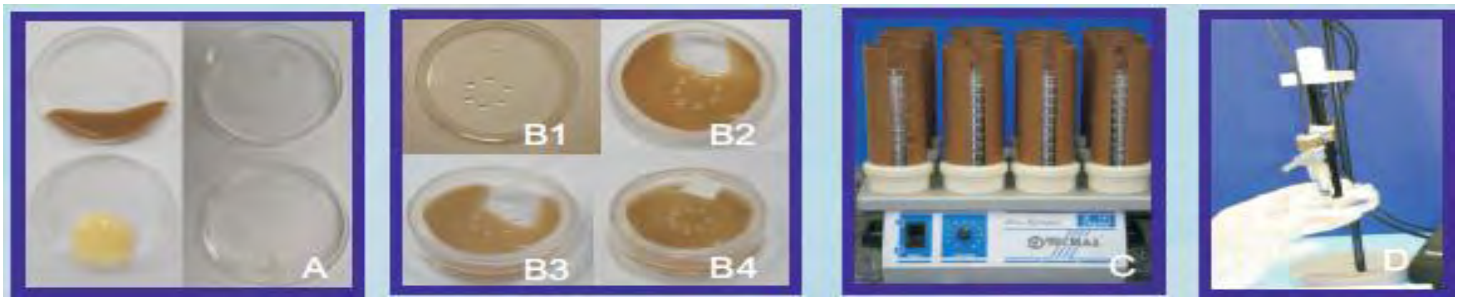
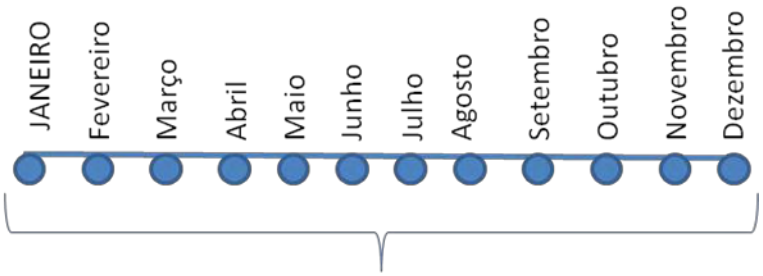


Esquematização para coleta de unnhas para análise da concentração de F presente nas unhas dos pés e mãos dos voluntários do estudo.



Coletadas 1 vez ao mês Unhas dos pés e mãos

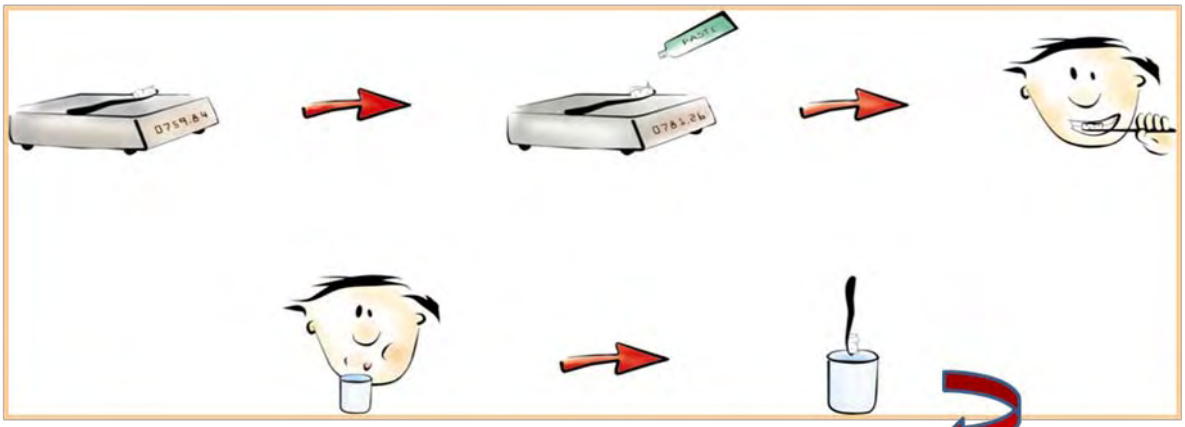
As concentrações de F das amostras das unhas após a lavagem e secagem foram analisadas após adição de hexametildisiloxano durante a noite (HMDS) difusão facilitada , utilizando um eletrodo flúor-sensível e um de referência de calomelano,

PROCEDIMENTO ANALÍTICO

Difusão facilitada por HMDS (Whitford, 1996)

Esquematização para análise da concentração de F ingerido apartir da escovação dos voluntários do estudo.

Estimativa da ingestão de flúor pelo dentifrício



As concentrações de F das amostras das expectoradas após uso do dentfrício em estudo, foram devidamente armazenadas para posterior analisadas após adição de hexametildisiloxano durante a noite (HMDS) difusão facilitada , utilizando um eletrodo flúor-sensível e um de referência de calomelano,

PROCEDIMENTO ANALÍTICO
Difusão facilitada por HMDS