CAROLINA DE CASTRO MARTINS

INGESTÃO E EXCREÇÃO URINÁRIA DE FLUORETOS ATRAVÉS DA ÁGUA E DO DENTIFRÍCIO

Belo Horizonte
Faculdade de Odontologia da UFMG
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Área de concentração: Odontopediatria

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"Comece fazendo o que é necessário,

depois o que é possível,

e de repente você estará

fazendo o impossível."

São Francisco de Assis

RESUMO

Ingestão e excreção urinária de fluoretos através da água e do dentifrício

RESUMO

Há poucos dados na literatura sobre o tempo necessário para que as crianças pré-escolares eliminem o fluoreto (F) circulante ingerido diariamente através da água e dos dentifrícios, bem como, se há concordância entre o hábito de escovação realizado por crianças, e o relato das mães sobre escovação dental. Sendo assim, o presente estudo, apresentado na forma de dois artigos científicos, teve como objetivos: 1) avaliar o efeito da interrupção de ingestão de fluoretos através da água e do dentifrício na excreção urinária de crianças; e 2) comparar a escovação observada por um pesquisador com o relado de mães sobre a escovação dos filhos. No artigo 1, foi selecionada uma amostra de conveniência de 11 crianças, residentes em Ibiá, MG, Brasil (água com concentração de flúor sub-ótima) com idade entre 2 a 4 anos, que usavam regularmente dentifrícios fluoretados. Foi feita coleta de urina 24 horas num dia 0 (baseline) (água fluoretada + dentifrício fluoretado); após a interrupção de F na água e no dentifrício (dias 1 a 28), e quando a ingestão de F a partir destas fontes foi re-estabelecida (dias 29 a 34). O volume urinário foi medido, a concentração de fluoretos foi determinada (mg F excretado /dia), e o teste de Wilcoxon foi usado para comparar as médias de excreção urinária de fluoretos (EUF) entre os dias ($p \leq 0,05$). No artigo 2, 201 mães de crianças pré-escolares (24 a 48 meses) de Montes Claros, MG, Brasil responderam um questionário autoadministrado sobre os hábitos de escovação de seus filhos. Em seguida, foi pedido para cada par de mãe/ criança realizasse a escovação, como usualmente é feita em casa. Os dados foram anotados por um observador. A escovação observada e a escovação relatada pelas mães foram comparadas para concordância geral, concordância pelo coeficiente de Cohen Kappa, teste qui-quadrado e teste exato de Fisher. O estudo 1 mostrou que o F foi excretado na urina em 24 horas: de 0,25 mg F/dia (± 0,15) em baseline para uma média de 0,14 mg F/dia nos dias 1 a 28 (± 0,07), diferença estatisticamente significativa ($p < 0,05$); e aumentou para 0,21 (± 0,09) no dia 32 e 0,19 (± 0,08) no dia 34, diferença não estatisticamente significativa com o baseline ($p > 0,05$). O estudo 2 mostrou que, no

geral, os valores de Kappa comparando o relato das mães com a escovação observada variaram de ruim a bom (0 a 0,75), com diferença estatisticamente significativa entre os dois métodos ($p < 0,05$). Os achados indicam que a excreção de F pela urina ocorre poucas horas após a sua ingestão; e a baixa concordância entre o relato das mães e os hábitos de escovação observados sugere que os questionários devem ser avaliados com cuidado para pesquisas epidemiológicas usando dentifrícios fluoretados e risco de desenvolvimento de fluorose dental.

Descritores: Fluoretos; Dentifrícios; Urina; Criança; Fluorose Dentária; Absorção; Farmacocinética.

ABSTRACT

Fluoride intake and urinary excretion from water and dentifrice

ABSTRACT

There are few data on how long it takes for children to eliminate circulating fluoride (F) acquired from the daily intake of water and dentifrice and whether there is agreement between observed tooth brushing habits of young children and those reported by mothers. The present study is comprised of two manuscripts, the aims of which were: 1) to investigate the effect of the discontinuation of fluoride intake from water and dentifrice on urinary fluoride excretion; and 2) to compare observed tooth brushing habits of young children and those reported by mothers. In the first paper, a convenience sample of 11 children from Ibiá, MG, Brazil (sub-optimally fluoridated water) aged from two to four years who regularly used fluoridated dentifrice took part in the study. Twenty-four-hour urine was collected from the children at baseline (Day 0) (water + fluoridated dentifrice), after the interruption of fluoride intake from water and dentifrice (Days 1 to 28) and after fluoride intake from these sources had been re-established (Days 29 to 34). Urinary volume was measured, fluoride concentration was determined and the amount of fluoride excreted was calculated (mg F/day). The Wilcoxon test was used to compare mean urinary fluoride excretion (UFE) between days ($p \leq 0.05$). In the 2nd paper, 201 mothers from Montes Claros, MG, Brazil answered a self-administered questionnaire on their child's tooth brushing habits. Each mother/children pair was then asked to perform the tooth brushing as they usually do at home. An examiner observed and documented the tooth brushing. Observed tooth brushing and that reported by mothers were compared for overall agreement using Cohen's Kappa coefficient, chi-square test and Fisher's exact test. The 1st study revealed that F was excreted through urine within 24 hours and remained stable over the subsequent days. Mean UFE at baseline was 0.25 mg F/day (± 0.15), dropping to a mean value of 0.14 mg F/day on Days 1 to 28 (± 0.07) (statistically significant difference, $p < 0.05$) and rising to 0.21 (± 0.09) on Day 32 and 0.19 (± 0.08) on Day 34 (no statistically significant difference, $p > 0.05$). In the 2nd study, Cohen's Kappa values

ranged from poor to good (0 to 0.75) when comparing mothers' reports and observed tooth brushing (statistically significant difference between methods, $p < 0.05$). The findings suggest that circulating F in the body of young children stemming from water and dentifrice intake is eliminated within a few hours after the discontinuation of the exposure. The low agreement between observed tooth brushing and the mothers' reports suggests that questionnaires should be considered with caution in epidemiological surveys on fluoridated dentifrice use and the risk of dental fluorosis.

Descriptors: Fluorides; Dentifrices; Urine; Child; Dental Fluorosis; Absorption; Pharmacokinetics.

LISTA DE ABREVIATURAS

COEP	Comitê de Ética em Pesquisa
DP	desvio padrão
EUF	excreção urinária de fluoretos
F	Fluoreto
F	*fluoride*
h	Horas
h	*hour*
MG	Minas Gerais
mg F/dia	miligramas de flúor por dia
mg F/day	*milligrams of fluoride per day*
mg F/Kg peso/dia	miligramas de flúor por quilo de peso corporal por dia
mg F/Kg body weight/day	*milligrams of fluoride per kilo body weight per day*
ml	Mililitro
ml	*milliliters*
ppm F	partes por milhão de flúor
ppm F	*parts per million of fluoride*
SD	*standard deviation*
SP	São Paulo
UFE	*urinary fluoride excretion*

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CONSIDERAÇÕES INICIAIS

CONSIDERAÇÕES INICIAIS

Os fluoretos foram amplamente difundidos nos últimos anos com o intuito de controlar a cárie dental. No entanto, o controle da cárie dental em populações tem sido acompanhado pelo aumento da prevalência de fluorose dental (Fomon *et al*., 2000; Buzalaf *et al*., 2001; Cury *et al*., 2004).

Os primeiros relatos de fluorose dental severa estiveram relacionados com a ingestão de água com altas concentrações de fluoreto (F) em diversas partes do mundo (Dean e Arnold, 1943; Thylstrup e Fejerskov, 1978; Paiva e Barros-Filho, 1993). Posteriormente, com o estabelecimento de que a água de abastecimento público deveria apresentar níveis ótimos de fluoretos, a fim de promover o máximo de benefícios contra a cárie dental, observou-se uma prevalência de fluorose dental em graus muito leve e leve (Leverett, 1986; Clark *et al*., 1994; Lalumandier e Rozier, 1995; Villa *et al*., 1998; Sampaio *et al*., 1999; Wondwossen *et al*., 2004; Catani *et al*., 2007).

A partir da década de 1980, outras fontes de fluoretos estiveram relacionadas com a ocorrência de fluorose dental muito leve, como suplementos fluoretados, dentifrícios fluoretados, géis, soluções tópicas para bochechos, chás, fórmulas infantis industrializadas e a própria dieta padrão (Osuji *et al*., 1988; Winkle *et al.*, 1995, Behrendt *et al*., 2002; Marinho *et al*., 2004; Casarin *et al*., 2007; Hujoel *et al*., 2009). Atualmente, o uso de dentifrícios fluoretados tem sido considerado fator de risco para a ocorrência de fluorose em comunidades com água de abastecimento público fluoretada, devido ao uso precoce por crianças que não conseguem cuspir a pasta, além do aumento da disponibilidade de dentifrícios (Cury e Tabchoury, 2003; Cury *et al*., 2004).

Os estudos sobre a ingestão de fluoretos por crianças pré-escolares, realizados em países desenvolvidos e em desenvolvimento, estimam que cerca de 20 a 45% da dose diária de ingestão de F é proveniente da dieta, e que 55 a 80% é em decorrência da ingestão de dentifrício fluoretado durante a escovação dos dentes (Guha-

Chowdhury *et al*., 1990; Guha-Chowdhury *et al*., 1996; Paiva *et al*., 2003; Franco *et al*., 2005; Almeida *et al*., 2007). Muitas vezes, a dose diária de ingestão de F está próxima ou acima da dose considerada limite pela literatura (Guha-Chowdhury *et al*., 1990; Guha-Chowdhury *et al*., 1996; Levy *et al*., 2001; Paiva *et al*., 2003; Villa *et al*., 1998; Franco *et al*., 2005; Oliveira *et al*., 2007; de Almeida *et al*., 2007). Alguns estudos observaram uma relação entre a dose de ingestão de F através dos dentifrícios fluoretados com a ocorrência de fluorose dental em incisivos permanentes (Franzman *et al*., 2006; Hong *et al*., 2006a,b), enquanto que outros não (Martins *et al*., 2008a).

A dose de exposição ao F que estaria relacionada à ocorrência de fluorose dental em crianças em idade de risco ainda não está clara. Uma das primeiras estimativas desta dose foi realizada por McClure (1943). Segundo o autor, uma dieta diária de 1,0 a 1,5 mg de F seria capaz de proporcionar uma exposição diária ao F de 0,05 mg F/Kg de peso em crianças de 1 a 12 anos. Anos mais tarde, Ophaug *et al*. (1980) afirmaram que a dose de 0,05-0,07 mg F/Kg de peso/dia seria a dose ótima. Este valor foi reafirmado por Burt (1992). Ficou estabelecido, até então, que a dose de 0,05-0,07 mg F/Kg de peso/dia seria a dose limite em termos de desenvolvimento de fluorose dental esteticamente comprometedora.

Os estudos sobre metabolismo do F no organismo são escassos devido à dificuldade de realização das metodologias. Muitas vezes, é necessária a coleta de unhas, sangue, saliva e urina. Estas substâncias podem ser difíceis de coletar, como a urina, a unha, que não se mostrou um biomarcador confiável para a ingestão de F através dos dentifrícios (Lima-Arsati *et al*., 2010), ou o sangue, que hoje não é prático por questões éticas.

A biodisponibilidade do F no organismo é rápida após sua ingestão. Sabe-se que após a ingestão de F via oral, o pico deste elemento no plasma ocorre cerca de 30 minutos após a ingestão do mesmo (Spak *et al*., 1982); no sangue ocorre após cerca de 40 a 50 minutos; e na saliva, ocorre após entre 45 a 55 minutos (Oliveby *et al*., 1989; Cury *et al*., 2005).

No entanto, nem todo F ingerido é absorvido pelo organismo. A absorção do F no organismo tem relação com o conteúdo estomacal. A absorção de F é menor quando a ingestão de F ocorre junto ou após a ingestão de leite, devido a reações entre os íons cálcio com o íon F (Spak *et al*., 1982; Trautner e Einwag, 1989). Após o almoço, a redução de absorção de F variou de 35 a 39%, sendo que após o café da manhã a variação foi de 22 a 28% (Cury *et al*., 2005). Por outro lado, a absorção de F após jejum é maior que quando realizada após as refeições (Drummond *et al*., 1990; Cury *et al*., 2005). Quando se estima a ingestão de F pela dieta e dentifrícios, através da coleta da dieta duplicada e da coleta dos resíduos da escovação (Guha-Chowdhury *et al*., 1990; Guha-Chowdhury *et al*., 1996), subtraindo os valores de excreção de F pela urina através da coleta da urina 24 horas, é possível estimar a retenção do F no organismo. A excreção do F no organismo é feita prioritariamente pela urina, sendo que alguns estudos que avaliaram a excreção urinária de fluoretos (EUF) em crianças observaram que a excreção variou de 32% a 80% (Villa *et al*., 2000; Zohouri e Rugg-Gunn, 2000; Haftenberger *et al*., 2001; Maguire *et al*., 2007), enquanto que a retenção estimada de F variou de 30,1% a 58% (Ketley e Lennon, 2000; Villa *et al*., 2000; Maguire *et al*., 2007).

Grande parte dos estudos avaliou a EUF em condições normais, ou seja, mediante ingestão de F pelo dentifrício e/ou dieta. A média de EUF variou de 0,2 mg F/dia em crianças moradoras de comunidade com 0,08 ppm F na água (não fluoretada) (Maguire *et al*. 2007); 0,2 a 0,36 mg F/dia quando crianças moravam em comunidades com 0,35 ppm F a 0,58 ppm F na água (água com F em nível sub-ótimo) (Zohouri e Rugg-Gunn, 2000; Villa *et al*., 2000); e entre 0,33 a 0,51 mg F/dia em crianças moradoras de comunidades com 0,8 ppm F na água (concentração ótima) (Ketley *et al*., 2004; Zohouri *et al*., 2006). Há evidências de uma relação entre o aumento da EUF de acordo com o teor de F na água (Maguire *et al*., 2007; Forte *et al*., 2008).

No entanto, poucos estudos avaliaram a EUF após a diminuição ou interrupção da ingestão de F. Um deles foi conduzido em uma comunidade que inicialmente apresentava água com concentração de 8,0 ppm F, e a concentração de F na água foi reduzida para 0,7 ppm F. O teor de F na urina das crianças de 7 a 16 anos de idade caiu de um valor inicial de 6,5 para 4,9 ppm F após uma semana, e se manteve estável por 39 semanas (4,9-3,5 ppm F), alcançando um valor de 2,2 ppm F na 113ª semana (Likins *et al*., 1956). Em outro estudo, crianças foram mantidas sem F na dieta e usaram dentifrício não fluoretado por 3 dias, e nos 2 dias subsequentes, as crianças ingeriram 0,5 mg F diluído na água . A média de excreção de F na urina aumentou de 0,18 mg para 0,32 F/dia (Ketley e Lennon, 2000).

Como pode ser observado, os estudos são pouco padronizados sobre a estimativa de excreção de F na urina, e poucos deles avaliaram as consequências da interrupção da ingestão de F por crianças (Likins *et al*., 1956; Ketley e Lennon, 2000). Pouco se sabe sobre quanto tempo demora para que a maior parte do F seja excretado pela urina após a interrupção da ingestão de F, principalmente considerando as principais fontes de exposição sistêmica de F, que são água e o dentifrício fluoretado. Após a interrupção da ingestão do F, a excreção urinária decresce até atingir um equilíbrio, sem sofrer grandes alterações de sua excreção, o chamado período *wash out*. É importante determinar o período *wash out* para a EUF no organismo, para que se possa planejar estudos que avaliem a EUF através do uso de dentifrícios com diferentes concentrações de F e através da água.

A água e o dentifrício são muito pesquisados para estimar a dose de exposição ao F por crianças, que estão em fase de risco para o desenvolvimento de fluorose dental (Guha-Chowdhury *et al.,* 1996; Paiva *et al*., 2003; Oliveira *et al*., 2007, Martins *et al*., 2008a). No entanto, os métodos de dieta duplicada e coleta dos resíduos da escovação, propostos por Guha-Chowdhury *et al*. (1990, 1996), necessitam da colaboração dos pais e das famílias para a coleta dos dados. Um dos requisitos fundamentais torna-se a seleção de voluntários colaborativos para participação da

pesquisa, no intuito de reduzir o viés. Existe a possibilidade do viés de informação, em que os pais podem se confundir sobre como eles se comportam e sobre o que eles acreditam ser o comportamento correto (Choi e Pak, 2005). Também existe a possibilidade de viés de memória das mães em lembrar episódios que ocorreram alguns anos atrás, como o início da escovação dos dentes dos filhos, para avaliar fatos retrospectivos da escovação (Martins *et al*., 2008b).

Um estudo comparando o método da dieta duplicada e diários dietéticos fornecidos pelo relato dos pais mostrou que o diário dietético proporcionou significativamente maior ingestão de F que o método da dieta duplicada (Martinez-Mier *et al*., 2009). Desta forma, os hábitos relatados e superestimados pelas mães podem superestimar a dose de exposição ao F. O mesmo acontece quando a frequência de escovação relatada pelas mães é usada para calcular a dose de exposição ao F a partir da escovação com os dentifrícios fluoretados. Assim, torna-se importante também comparar o relato das mães sobre a escovação dos dentes dos filhos com o método observado.

O trabalho foi desenvolvido junto ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Minas Gerais, com colaboração da Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas. Optou-se pela apresentação da tese em forma de dois artigos científicos, por ser uma forma de divulgação objetiva e clara. O primeiro artigo apresentado se refere à EUF após a interrupção do uso do F. O local da pesquisa foi à cidade de Ibiá, MG, onde os dados foram todos coletados. Fez-se opção por Ibiá por já ter sido sede de trabalhos desenvolvidos pela Faculdade de Odontologia da UFMG em 1998 e 2004 (Paiva *et al*., 2003; Martins *et al*., 2008a). Além de ser uma cidade de pequeno porte, fácil acesso e com uma população receptiva para a realização da pesquisa. Todas as análises químicas dos dados coletados foram realizadas na Faculdade de Odontologia de Piracicaba da UNICAMP.

O segundo artigo apresentado comparou o método da coleta dos resíduos da escovação com o relato de mães sobre os hábitos de higiene bucal dos filhos. A coleta foi realizada na cidade de Montes Claros, por ser sede de uma pesquisa transversal (Oliveira *et al.*, 2007) sobre exposição ao F por crianças comparando o dentifrício infantil e o regular.

ARTIGO 1

Effect of discontinuation of fluoride intake from water and dentifrice on urinary excretion in young children

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Abstract

As there is no homeostatic mechanism for maintaining circulating fluoride (F) in the human body, the concentration may decrease and increase again when intake is interrupted and re-started. The present study prospectively evaluated this process in children exposed to F intake from water and dentifrice, using F in urine as a biomarker. Eleven children from Ibiá, Brazil (with sub-optimally fluoridated water supply) aged two to four years who regularly used fluoridated dentifrice (1100 ppm F) took part in the study. Twenty-four-hour urine was collected at baseline (Day 0) (F exposure from water and dentifrice) as well as after the interruption of fluoride intake from water and dentifrice (Days 1 to 28) (F interruption) and after fluoride intake from these sources had been re-established (Days 29 to 34) (F re-exposure). Urinary volume was measured, fluoride concentration was determined and the amount of fluoride excreted was calculated and expressed in mg F/day. Urinary fluoride excretion (UFE) during the periods of fluoride exposure, interruption and re-exposure was analyzed using the Wilcoxon test. Mean UFE was 0.25 mg F/day (SD: 0.15) at baseline, dropped to a mean of 0.14 mg F/day during F interruption (SD: 0.07; range: 0.11 to 0.17 mg F/day) and rose to 0.21 (SD: 0.09) and 0.19 (SD: 0.08) following F re-exposure. The difference between baseline UFE and the period of F interruption was statistically significant ($p < 0.05$), while the difference between baseline and the period of F re-exposure was non-significant ($p > 0.05$). The findings suggest that circulating F in the body of young children is eliminated in about 24 hours following F discontinuation and increases very quickly after F re-exposure from water and dentifrice.

Keywords: fluoride; dentifrice; urine; drinking water.

1. Introduction

There has been an increase in the search for biomarkers for monitoring different sources of fluoride (F) intake [1], including fingernails [2, 3] and urine [4]. Among the sources F intake, drinking water and dentifrices are considered risk factors of fluorosis [5], although the real contribution of each source to the development of fluorosis is not clear and a dose-response effect has not yet been established [6].

The use of urine as potential biomarker of F intake from diet and fluoridated dentifrices has been investigated [7-12]. These studies have an observational design and evaluate F intake from water and dentifrices at a single point in time, with no experimental data and no follow up of the individuals. Thus, there is a lack of follow-up studies aimed at evaluating the variation in urinary fluoride excretion (UFE) when F is discontinued. Knowledge on the role of urine as a biomarker of F is important to providing data on the amount of F ingested by children at ages of risk for the development of dental fluorosis.

UFE under different concentrations of F in water and dentifrice over time should also be evaluated. There is insufficient scientific evidence to indicate how many days it takes UFE to decrease and remain stable after F intake is discontinued. Moreover, there is a lack of methodological standardization in studies investigating UFE following the discontinuation of F intake. Such studies have comprised individuals in different age groups [13, 14], with different sources of F, such as in supplements [14, 15] or milk [15], and different F concentrations [13]. Considering the lack of scientific evidence on the effect of the discontinuation of F from water and dentifrice, it is important to investigate the release of F retained following the discontinuation of intake and the effect after F re-exposure under controlled conditions, using urine as a biomarker. Thus, the aim of the present study was to carry out a prospective investigation into the effect of the discontinuation of F intake from the water supply and fluoridated dentifrice.

2. Experimental Section

The present study received approval from the Ethics Committee of the Federal University of Minas Gerais (process number: 279/07). All parents/guardians received information regarding the objectives of the study and signed terms of informed consent.

2.1. Subjects

Eleven healthy children (six girls and five boys) aged two to four years (mean age: 43.9 months; 34 to 56 months) participated in the present prospective study in May and June 2008. The investigation took place in the city of Ibiá, MG, Brazil. All children had lived in Ibiá since birth. Water samples were collected from the public water supply, which was determined to be sub-optimally fluoridated.

Children were recruited at a private day care centre. The following were the inclusion criteria: good systemic health status; good oral health status; age between two to four years; absence of medications or fluoridated supplements; and very high parent compliance with the study protocol. Parents were interviewed regarding their children's overall health, tooth brushing habits, diet and use of fluoridated supplements. The main fluoride sources were fluoridated dentifrice and public drinking water. All children regularly used a Tandy® dentifrice, which is a fruit flavoured children's toothpaste with fluoride concentration declared by the manufacturer of 1100 ppm F, sodium fluoride and silica as the abrasive (Tandy®, Colgate/Palmolive Ind. Ltda, São Bernardo do Campo, SP, Brazil). All children were weighed prior to the beginning of the study (mean weight: 17.6 kg ± 4.74).

2.2. Experimental design

Twenty-four-hour urine was collected to determine urinary fluoride excretion (UFE) under normal conditions (baseline – Day 0). The children were then refrained from fluoride intake from water and dentifrice until Day 28 (F interruption). Twenty-four-

hour urine was collected during F interruption on Days 1, 2, 4, 8, 12, 16, 20, 24 and 28 (Figure 1).

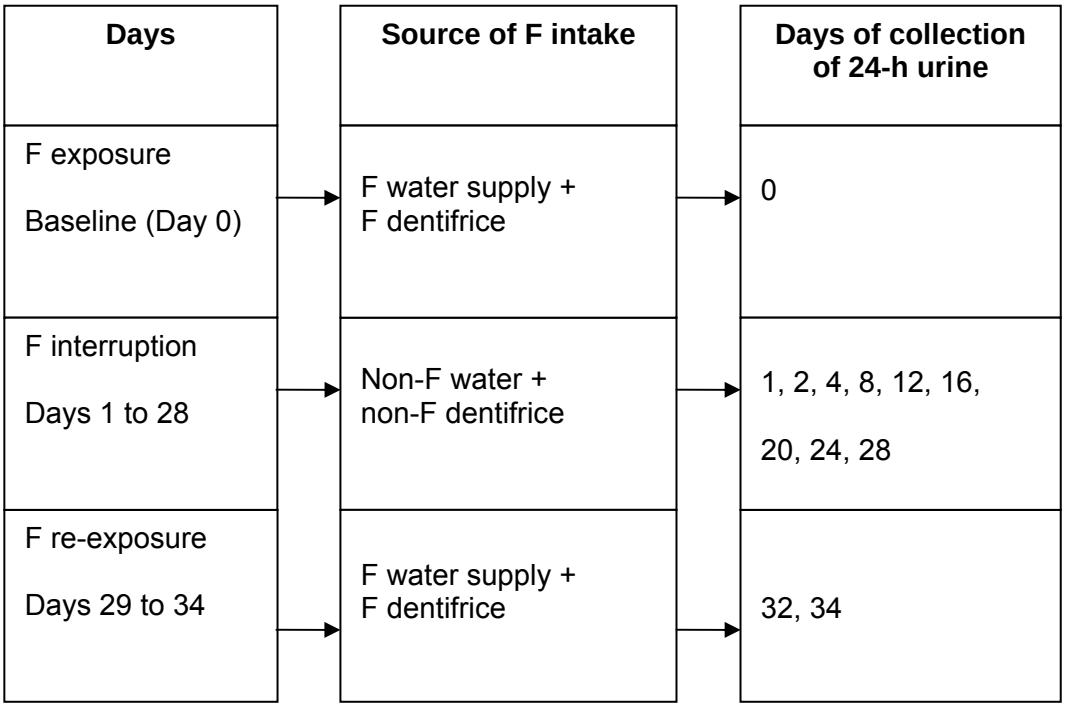

Figure 1: Illustration of experimental design

From Days 1 to 28, the children were supplied with de-ionized water prepared for the study at a laboratory (Laboratório Vita Center, Ibiá, MG, Brazil) and the families were given a daily supply of five-litre bottles. The de-ionized water was analyzed for fluoride content at the Piracicaba Dental School of the University of Campinas (Brazil) and had a mean concentration < 0.01 ppm F. The parents were carefully instructed that all drinks, juices, food, soups and powdered milk were to be prepared with this water. It was stressed that the de-ionized water was to be the only water that the children would have throughout this phase of the experiment. Parents were instructed that children should avoid teas and any kind of seafood, as some of these items can have considerable F concentrations [16].

The children were also supplied with a non-fluoridated dentifrice (Malvatri Kids baby®, Laboratório Daudt Oliveira Ltda, Rio de Janeiro, RJ, Brazil) and a children's toothbrush. The parents were instructed that the children were to use only this dentifrice during the 28-day period at a frequency of three times per day. This frequency was intended to introduce a routine for oral hygiene in the children. No other dentifrice or other topical fluoride was to be used during this phase of the experiment.

All volunteers were recruited from the same day care centre in order to facilitate the water control. The day care centre was given a daily supply of de-ionized water. The children enrolled in the study had day care in the afternoon. The afternoon snack was prepared at home. After recreation, the children usually brushed their teeth. Therefore, the day care staff members received the non-fluoridated dentifrice and were instructed to use only this dentifrice during this phase of the experiment. The experiment was carefully supervised by one of the researchers, who was present at the day care centre every day. The deionized water was delivered every morning by the same researcher, at which time the instructions were reinforced and the research protocol could be monitored.

On Day 29, the parents were instructed to re-establish the previous fluoride conditions (F re-exposure). The non-fluoridated water regimen was suspended and the parents received a fluoridated dentifrice to be used by the children when brushing (Tandy®, 1100 ppm F, Colgate/Palmolive Ind. Ltda, São Bernardo do Campo, SP, Brazil). The day care staff received the same dentifrice and the drinking water went back to being from the public water supply. Twenty-four-hour urine was collected under fluoridated conditions on Days 32 and 34.

2.3. Collection of 24-hour urine

Urine was collected in 24-h periods. The children were provided with a two-litre plastic collection bottle with a screw top. Parents were instructed with regard to the importance of collecting all urine during the 24-h period. At the day care centre, the

urine was collected every time the children asked to go to the bathroom. The bottles were labelled with the children's names. The parents deposited the urine from the previous day at the day care centre on the scheduled days. The volume collected at home was mixed with the volume collected at the day care centre. The total volume was recorded and approximately 20 ml was placed in a sterile plastic cylinder containing thymol as a preservative (Amphora Farmácia de Manipulação Ltda, Belo Horizonte, MG, Brazil). The samples were frozen until analysis. Fluoride elimination via faeces and sweat was not assessed. The samples were sent to the Piracicaba Dental School of the University of Campinas and analyzed for fluoride content.

2.4. Collection of public water supply

Samples from the public water supply were collected for seven days prior to beginning the study. Samples from the public water supply were also collected from Days 29 to 34. All water samples were sent for analysis at the Piracicaba Dental School of the University of Campinas.

2.5. Fluoride analysis

Fluoride was analyzed in duplicate aliquots buffered with TISAB II using an ion-selective electrode (Orion 96-09, Orion Research, Cambridge, Mass, USA) and ion analyzer (Orion EA-940), which were previously calibrated with standard F solutions (Orion 940907). Analysis was validated using internal standards and a coefficient variation lower than 3% was considered acceptable.

2.6. Data analysis

Descriptive analysis was carried out. The quantitative variables were tested for normality using the Kolmogorov-Smirnov test. Levene's test was used to determine the homogeneity of the variance. As the data had non-normal distribution and variance was not homogeneous, the non-parametric Friedman and Wilcoxon tests were used to

determine statistically significant differences in fluoride urinary excretion values between evaluations. Statistical significance was set at 0.05.

3. Results

Mean 24-hour urinary volume from baseline (Day 0) to Day 34 was 398.11 ± 231.97 ml (minimum: 296.67 ± 172.34 ml; maximum: 471.36 ± 353.66 ml). There were no statistically significant differences in mean urinary volume between evaluations ($p > 0.05$).

Figure 2 displays the mean 24-h urinary fluoride excretion (UFE) over time. At baseline, fluoride excretion was 0.25 mg F/day (SD: 0.15), which rapidly decreased to 0.14 mg F/day on Day 1. Fluoride excretion then varied little through to Day 28, maintaining a mean of 0.14 ± 0.07 mg F/day. UFE rose again on Days 32 (0.21 ± 0.09 mg F/day) and 34 (0.19 ± 0.08 mg F/day), but did not reach the baseline value. The Friedman test revealed a significant difference between Days 0 to 34 ($p = 0.013$). There was a statistically significant difference in UFE between baseline (0.25 mg F/day) and the mean value of the period from Day 1 to 28 (0.14 mg F/day) (Wilcoxon test, $p < 0.05$). The Wilcoxon test also revealed significant differences between baseline and all days following F interruption ($p < 0.05$, represented in Figure 2 by different letters).

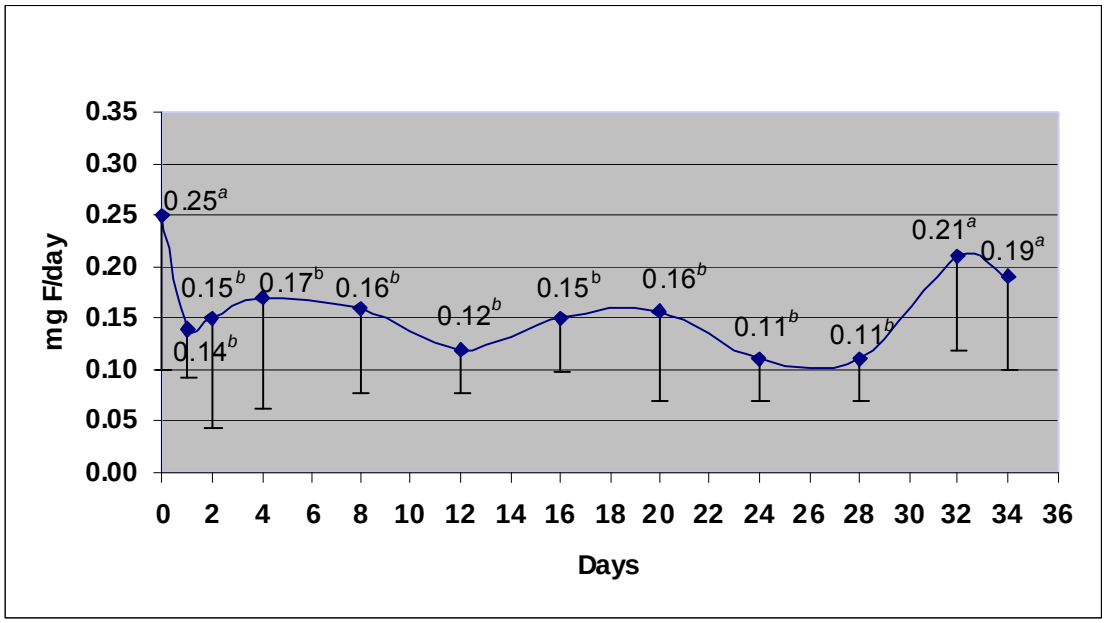

Figure 2: Mean urinary fluoride excretion (mg F/day) at baseline (Day 0), after interruption of fluoride intake from water and dentifrice (Days 1 to 28) and after fluoride intake had been re-established (Days 29 to 34); statistically significant difference in mean UFE between Days 0 to 34 ($p = 0.013$, Friedman test); means followed by different letters are statistically different (Wilcoxon test; $p \leq 0.05$); bars represent standard deviation (n=11)

Fluoride content in the samples of the public water supply was analyzed for seven days prior to beginning the study. The water was sub-optimally fluoridated (mean: 0.2 ppm F). When the fluoride intake was re-established (Days 29 to 34), the levels of fluoride in the drinking water had rose to 0.4 ppm F.

Table 1 displays UFE values at baseline, during the period of F interruption and Days 32 and 34. The values for Days 32 and 34 were adjusted as if the water had maintained a constant level of 0.2 ppm F throughout the entire study. These adjusted values were 0.17 and 0.16 mg F/day, respectively. As 35% of fluoride intake is from water and 65% is from brushing with a fluoridated dentifrice [17] and considering the positive correlation between fluoride intake and urinary fluoride excretion [9, 12], 0.07 mg F/day of the value excreted on Day 32 was from water intake (35% of 0.21 mg

F/day = 0.07) and 0.14 mg F was from dentifrice intake (65% of 0.12 mg F/day = 0.14). As the water had twofold more fluoride on Days 32 and 34 (0.4 ppm F) than at baseline (0.2 ppm F), the children would have ingested half the amount of fluoride from water on these days if the content were at 0.2 ppm F and would have excreted half the fluoride from water intake (half of 35%, which is ½ of 0.07 = 0.035 mg F/day). Thus, adding 0.035 mg F/day (excreted due to water intake) to 0.14 mg F/day (excreted due to dentifrice intake), the final adjusted value is 0.17 mg F/day of fluoride excreted on Day 32 if the water had had a F content of 0.2 ppm. The same calculation was made for Day 34. Considering the adjusted values, UFE at baseline (0.25 mg F/day) was significantly different from Days 1 to 28 (0.14 mg F/day) as well as Days 32 (0.17 mg F/day) and 34 (0.19 mg F/day) ($p \leq 0.05$) and there were no statistically significant differences between Days 1 to 28 and Days 32 and 34 ($p > 0.05$).

Table 1: Mean (±SD; n=11) amount of fluoride (mg F/day) in urine of children from Days 0 to 34 and adjusted values based on fluoride intake

Days	Source of F intake	mg F/day	
		Found	Adjusted
F exposure (0)	Water+Dentifrice	0.25 ± 0.15 [a]	0.25 ± 0.15 [a]
F interruption (1 to 28)	None	0.14 ± 0.02 [b]	0.14 ± 0.02 [b]
F re-exposure (32)	Water+Dentifrice	0.21 ± 0.09 [a]	0.17 ± 0.07* [b]
(34)	Water+Dentifrice	0.19 ± 0.08 [a,b]	0.16 ± 0.06* [b]

*Means adjusted considering water at 0.2 ppm F

[a,b] Means (within columns) followed by different letters are statistically different (Wilcoxon test; $p \leq 0.05$)

4. Discussion

Twenty-four-hour urine sampling is a reliable method and measures the fluoride content of an entire day. However, it requires a very high degree of family compliance and there is a risk of sample loss. In order to minimize such loss, the families were carefully prepared and selected in order to achieve maximal cooperation. The effort on the part of the parents to cooperate with the study is evidenced by the little variation in mean urinary volume throughout the study (mean: 398.11 ± 231.97 ml).

Urinary fluoride excretion at baseline (Day 0) was 0.25 mg F/day (SD: 0.15) and fluoride content in the public water supply was 0.2 ppm, which is close to the values found in the literature on UFE under sub-optimal water fluoride conditions (0.20 to 0.39 mg F/day for water with ≥ 0.15 to < 0.7 ppm F) [8, 9, 11, 12].

The data suggest that urine as a biomarker can rapidly detect variations in F intake and stabilization was achieved in about 24 hours. This is in agreement with the literature, which reports that fluoride is excreted from the body in the hours following its intake from fluoridated salt, fluoridated dentifrice [18] and fluoridated water [19]. When fluoride conditions were re-established, UFE increased again. This finding corroborates the results of a previous study, in which UFE rose from 0.18 to 0.33 mg F/day when children refrained from ingesting fluoride for three days and then received a 0.5-mg F tablet with half glass of water over the subsequent two days [15]. One may speculate that if the period of F interruption were longer, UFE would continue to decline over time, albeit very little. A previous study reports that UFE continued to exhibit a slight decrease even after 113 weeks; however, subjects had been chronically exposed to an above-optimal water supply (8.0 ppm F) and the water was de-fluoridated to an optimal level (0.7 ppm F) [13].

The results may have been different if the experiment had been conducted with adults, as fluoride retention (percentage of absorbed fluoride), extrarenal clearance, skeletal uptake [20] and fluoride removal from the plasma is generally greater in

children than adults [21, 22]. This may explain the rapid decrease in fluoride excretion in the first 24 hours. A negative balance (when fluoride intake is less than fluoride excretion) may indicate that skeletal and perhaps dental stocks of fluoride are being depleted [22]. However, the rapid decrease in fluoride excretion in the present study did not indicate that skeletal and dental stocks were totally depleted, as UFE continued to be excreted throughout the period of F interruption, thereby indicating that exchanges between mineralized tissues and plasma continued to occur. Besides the F reservoir in the bones, UFE during the period of F interruption never achieved values near 0 due to the natural F content in meals [23].

When the fluoride conditions were re-established, UFE increased, but neither reached the baseline value nor remained stable, although the fluoride content in the water had increased twofold (to 0.4 ppm F). One hypothesis is that some mothers may have forgotten to use the fluoridated dentifrice at the recommended frequency. Another hypothesis is that it may take longer to achieve the balance (fluoride intake equal to fluoride excretion), which was not re-established after six days (Day 29 to 34). Following intake, fluoride is absorbed by the gastrointestinal tract, distributed through the blood to different tissues and incorporated in mineralized tissues. When the amount of fluoride surpasses the tissue uptake capacity, a plateau is reached and there is no further increase in fluoride incorporation. This situation creates a balance in the organism, in which fluoride uptake by bone is equal to fluoride excretion [24]. As fluoride excretion did not reach the baseline value in the present study, the balance had not yet been established and fluoride uptake in the bone was still occurring at the end of the experiment.

The balance between fluoride uptake and excretion is uncertain and may even take months. Two studies have evaluated the onset of salt and water fluoridation in two communities with no previous history of fluoride in the drinking water [25] or salt [26]. For children, it took 20 months to reach the balance after the onset of salt fluoridation [26] and approximately three years after the onset of water fluoridation, while the

balance in adults was achieved in one week [25]. This supports the notion that children require more time to reach a balance due the retention of F in the bones.

The children in Ibiá were found to be exposed to a F dose of 0.08 mg F/Kg/day from water and dentifrice [17]. Multiplied by the mean body weight of the children in the present study (17.59 ± 4.74 Kg), mean F intake was about 1.41 mg F/day. UFE was 0.25 mg F/day at baseline, which corresponds to 17.7% excretion of total F intake. Considering 10% excretion through faeces [27, 28], about 72.3% was retained by the children [100% – (17.7% + 10%) = 72.3%]. The higher UFE values reported in the literature (30.0% to 80.0%) [8-10, 12] and mean retention of 50 to 55% [8, 12] have been suggested to be inaccurate for lower F intake and actively growing young children, whose bone turnover is higher [20], as in the case of the children in the present study.

The present study has some limitations that should be considered when interpreting the results. Due to the complexity of the experimental design, a convenience sample was used and it was not possible to conduct the study with a larger sample. A single baseline measurement was performed and there was limited follow up after restarting the usual fluoride intake from water and dentifrice. Unfortunately, it was not possible to continue the study until reaching the fluoride balance, as the study was designed to last only one month in order to determine the decrease in fluoride excretion. The study was conducted under conditions of sub-optimally fluoridated drinking water and the fluoride content in the water varied throughout the study period. The results may have been different if the water had been optimally fluoridated. The results may also have been different if the study had been conducted in the summer, as fluoride intake by children may vary depending on the season [29]. In Brazil, May and June are late autumn months, when children may drink less water than in summer. Further studies should be performed to evaluate fluoride excretion with an optimally fluoridated water supply associated to fluoridated dentifrices.

5. Conclusion

The findings of the present study suggest that circulating F in the body of young children due to water and dentifrice intake is rapidly eliminated 24 hours following discontinuation of the exposure and increases very quickly again after F re-exposure.

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ARTIGO 2

Comparison between observed children's tooth brushing habits and those reported by mothers

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Abstract

Background: The aim of the present study was to compare observed tooth brushing habits of young children using fluoridated toothpaste and the habits reported by mothers.

Methods: Two hundred one mothers and their children (aged 24 to 48 months) from Montes Claros, Brazil, took part in a cross-sectional study. The mothers answered a self-administered questionnaire on their child's tooth brushing habits. The structured questionnaire had six items with two or three options. An appointment was then made with each mother/child pair at day care centres. The participants were asked to demonstrate the tooth brushing practice as usually performed at home. An examiner observed and documented the procedure. Observed tooth brushing and that reported by mothers were compared for overall agreement using Cohen's Kappa coefficient and the McNemar test.

Results: Cohen's Kappa values comparing mothers' reports and tooth brushing observed by the examiner ranged from poor to good (0 to 0.75). There were statistically significant differences between observed tooth brushing habits and those reported by the mothers ($p < 0.001$). When observed by the examiner, the frequencies of dentifrice dispersed on all bristles (35.9%), children who bushed their teeth alone (33.8%) and those who did not rinse their mouths during brushing (42.0%) were higher than those reported by the mothers (12.1%, 18.9% and 6.5%, respectively; $p < 0.001$).

Conclusions: In general, there was low agreement between observed brushing and mothers' reports. Moreover, the different methods employed determined different frequencies of habits, suggesting that children's tooth brushing habits as reported by mothers and determined through observation data should be considered with caution in epidemiological surveys on fluoridated dentifrice use and the risk of dental fluorosis.

Background

Information bias can occur in epidemiological studies and compromise the scientific outcome, especially when evaluating patient information regarding one's own health. The aim of the study design is to obtain the most accurate results to represent reality. In paediatric dentistry, a number of studies have evaluated fluoride intake among children from diet and tooth brushing with fluoridated dentifrice [1-4]. The current method used to evaluate fluoride intake from diet is the duplicate plate method [1]. A comparison between the duplicate plate method and dietary logs provided by parents' reports revealed differences in fluoride intake by children between methods, with the dietary log reporting significantly higher fluoride intake from food than the duplicate plate method [5]. However, the few other studies comparing methodologies for food and beverage consumption on the part of young children focus on the risk of obesity [6, 7].

To estimate the risk of dental fluorosis in young children, fluoride intake also considers tooth brushing with fluoridated dentifrices. Young children often ingest a large portion of the dentifrice dispersed on the toothbrush, thereby increasing the risk of developing dental fluorosis [3, 4]. One method for evaluating children's tooth brushing habits is through direct observation. The examiner observes without intervening or giving explanations and takes notes on the process. This method was used as the gold standard for comparison to another method in a previous study [8]. The use of mothers' reports regarding their children's tooth brushing habits is another commonly employed method. One study found statistically significant differences in mothers' reports regarding their children's brushing habits when the same interview was repeated six years later, suggesting memory bias [9]. However, there are a lack of studies determining whether mothers' reports are similar to the actual brushing performance of their children. As reliable data are important to the assessment of risk factors, information bias can compromise the final results. For example, the daily

frequency of tooth brushing is used to calculate a child's daily fluoride intake from dentifrice [3]. Thus, the over-reporting of daily tooth brushing frequency on the part of mothers leads to an overestimation of children's daily fluoride intake. Considering the lack of studies comparing information collected using different methodologies, it is important to evaluate these data in order to determine the most reliable, most valid method.

The aim of the present study was to compare agreement between observed children's tooth brushing habits using fluoridated toothpastes with the habits reported by mothers.

Methods

The present study was conducted as part of a larger cross-sectional study on fluoride intake by dentifrices among children from Montes Claros, MG, Brazil. Eight day care centres in the city of Montes Claros were randomly selected from a list of day care centres compiled by the Municipal Department of Education. The directors of the centres were contacted and gave consent for the conduction of the study. A meeting was initially set up with parents, at which time the mothers received information on the objectives and signed terms of informed consent authorising participation in the study. It was made clear that the presence of mothers was preferred over fathers and, thus, only mothers were present at the meeting. The following were the inclusion criteria to take part in the study: mothers must be present at the meeting and their children must be between 24 to 48 months of age. Five mothers had children less than 24 months of age and were excluded from the study. The initial sample was comprised of 203 mothers, among whom two failed to complete the questionnaire and were excluded from the study. The final sample was comprised of 201 pairs of mothers and children (0.98% of drop-outs). The mean age of the children was 41.3 months. The sample was selected by convenience.

Data collection was carried out from October 2007 to June 2008. The study received approval from the Human Research Ethics Committee of the Federal University of Minas Gerais (ETIC 278/07).

Mothers' reports

Mothers who agreed to participate by signing an informed consent form were then asked to answer a structured questionnaire on their children's current tooth brushing habits. A self-administered questionnaire was distributed by one of the examiners (MJO) and filled out by the mothers, who were instructed that there were no right or wrong answers and they should answer the questionnaire based on their children's tooth brushing habits at home. The questionnaire was structurally composed of six items – five with two options and one with three options (Table 1). The first item "kind of dentifrice the child uses" had two options, "children's" and "adults'". In Brazil, children's dentifrices are specially flavoured for children (fruit, gum, strawberry, grape, etc.) and usually contain between 0.0 to 1100 ppm F. Adults' dentifrices are mint flavoured and contain between 1100 to 1500 ppm F [10]. The brand names were maintained in the questionnaire in order to enhance understanding on the part of the respondents.

Tooth brushing observed by an examiner

In the second part of the study, an appointment was made with the mothers and their children at the day care centre one week after answering the questionnaire. The mothers were asked to bring the dentifrice and tooth brush that the child used at home. An appointment was made for each mother/child pair separately, without the presence of other mothers. The mothers and children were led to a bathroom and were asked to perform the tooth brushing, reproducing the same manner normally employed at home. No instructions on tooth brushing were given. An examiner (MJO) observed without intervening and recorded notes on a structured form, which contained the same items

and response options as the questionnaire (Table 1). The examiner maintained sufficient distance while observing so as not to disturb the normal routine of the process. The tooth brushing practice of each mother/child pair was only observed once.

The observation method has been used as the gold standard to evaluate another method in a previous study [8]. The examiner was an experienced paediatric dentist who underwent a training process for the observation of tooth brushing. The calibration process is described below.

Calibration process

Before the main study, a training process was conducted to ensure that the method would be understood by the participants. For such, a day care centre that was not part of the main sample was chosen. Ten mother/child pairs took part in this process. The mothers were asked to answer the questionnaire and all items were fully understood. Each mother/child pair was then asked to perform the tooth brushing at the day care centre. There were no major corrections required for the calibration process and the main study was then conducted. In this part of the study, the examiner was able to improve the observation method.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS for Windows, version 12.0, SPSS Inc., Chicago, IL, USA). Mothers' reports were compared to observed tooth brushing and the data were analysed for overall agreement (%) and Cohen's Kappa coefficient. To calculate the Kappa coefficient, the following formula was used: $k = \frac{po - pe}{1 - pe}$, in which po= is the proportion of units with agreement: $po = \frac{a + d}{a + b + c + d}$ and pe = is the proportion of units for which agreement

is expected by chance: $\frac{[(a+b).(a+c)]+[(c+d).(b+d)]}{(a+b+c+d)^2}$ [11]. Agreement strength was based on the following criteria: 0.00 to 0.20 = 'poor'; 0.21 to 0.40 = 'fair'; 0.41 to 0.60 = 'moderate'; 0.61 to 0.80 = 'good'; 0.81 to 1.00 = 'very good' [12]. Overall agreement considers the proportion of total agreement divided by the total (po) and not by chance (Kappa). The McNemar test was used to compare the frequencies of tooth brushing habits between mothers' reports and those observed by the examiner (level of significance set at 5%). Missing data were "I don't know" answers and those left blank on the questionnaire, which were not considered in the analyses.

Results

Eighty-nine children were male (44.3%) and 112 were female (55.7%); 72 children were from private day care centres (35.8%) and 129 were from public day care centres (64.2%). Table 1 displays the comparison between the observed data and those from the mothers' reports.

Kappa agreement ranged from 0.00 to 0.75. The "kind of dentifrice the child uses" achieved the best agreement (good, K= 0.75, Standard Error= 0.05) and the highest overall agreement (87.6%). All other items achieved moderate to poor agreement (K= 0.43 to 0.00). Overall agreement ranged from 47.0 to 87.6%, which is actual agreement in proportion to total possible agreement. Table 1 also displays the agreement for each answer, the values of which are on the diagonal line. For example, 10 mothers reported that their child did not rinse his/her mouth during tooth brushing, which was also observed by the examiner (last question). One hundred thirteen mothers reported that their child rinsed his/her mouth during brushing, which was in agreement with the observed finding. For the remaining 77 answers, the observed data did not match those reported by the mothers.

Comparisons of frequencies between the observed results and mothers' reports were statistically different on all questions (McNemar test, $p < 0.05$) (Table 1). The frequencies of children's dentifrice use (54.7%), amount of dentifrice dispersed on all the bristles (35.9%) and children who spit the dentifrice out (78.2%) were significantly higher when observed by the examiner than when reported by mothers (49.3%, 12.1% and 39.6%, respectively; $p < 0.05$, McNemar test). In contrast, the frequencies of adults who brushed the child's teeth (81.1%) and children who rinsed their mouth out during brushing (93.5%) were significantly higher when reported by mothers than observed by the examiner (66.2% and 58.0%, respectively; $p < 0.001$).

Discussion

The most widely employed methods for evaluating patient-reported health conditions are interviews and questionnaires [13-16]. However, information from patients can be biased due to forgotten past episodes or the over-reporting of certain habits in order to appear careful about one's health.

In the present study, the majority of children were enrolled in public day care centres (64.2%) and the rest were enrolled in private day care centres (35.8%). These institutions mainly carried out teaching activities directed at preschool children. No institution offered a class in oral health. The education was similar in both groups of children. Thus, the oral health habits of these children were not influenced by the learning process at school.

Parental opinion is considered a valuable tool for the assessment of children's conditions. A previous Brazilian study tested the validity of mothers' opinions regarding their child's life [17]. The choice of collecting data at day care centres was based on the fact of many mothers leave their children at such centres while working rather than leaving them with nannies or grandparents. Moreover, it is possible to find all types of mothers at day care centres. Brazil is a predominantly catholic country with married,

divorced and single mothers. Many married woman work to help with family's expenses. Some have a lower salary than their husbands, whereas others have a higher salary. There are also married mothers who do not work and only take care of the family. In the case of divorced women, Brazilian law gives the woman the priority regarding the guardianship of the child. There is also a portion of single mothers who live with their parents or who are the head of the family.

A previous study carried out in Brazil demonstrated mothers' comprehension regarding their children's cognitive, psychological, emotional and physical development. The study also found that mothers consider their presence of great importance to raising and educating their children and consider their presence more important than that of the father in the tasks of care and education, whereas the presence of the father is considered important as a male role model [18].

Kappa values were mainly low, ranging from poor to moderate. Overall agreement was higher than the Kappa value in most cases, which corroborates the findings of previous studies [9, 19, 20]. The reason for this is that the Kappa index applies adjusted measures for random agreement when the same fact is evaluated twice [11]. The item "kind of dentifrice that the child uses" achieved the highest overall agreement and good Kappa agreement. This was perhaps the easiest question and it is likely that the mothers were responsible for buying the dentifrice, making it easy to remember. Other questions contained details that were harder to remember, such as whether the child spit the paste out and the amount of dentifrice used. Another factor that could have been related to the poor Kappa values on these two items is the fact that young children are still developing their tooth brushing habits and spitting the paste out and rinsing the mouth out can vary between brushings. Moreover, such children are generally not capable of spitting the paste out and rinsing properly and therefore ingest a large portion of dentifrice during tooth brushing [21]. However, these items were added to test whether the mothers were aware of the inability of their young children to spit paste out and rinse their mouths out. More mothers answered "no" than "yes" to

the item “does the child spit out the dentifrice during brushing?”, demonstrating that the mothers are aware of their children’s limited brushing skills. The item “does the child rinse out his/her mouth during brushing” also revealed that mothers do not have much knowledge regarding the fact that young children cannot properly perform this act, as nearly all the mothers answered “yes”.

There were statistically significant differences between reported habits and observed tooth brushing. A previous study also found differences in the reporting of skin health when evaluated through a telephone interview and mailed questionnaire. When patients were asked whether a non-doctor, such as a partner, had checked his/her skin over their entire body, the prevalence of patients who answered ‘yes’ for the same question was greater during the telephone interviews than on the mailed questionnaires ($p < 0.03$) [14]. However, for the determination of food and beverage intake for the assessment of the risk of obesity in young children, parents’ reports using a food consumption and physical activity questionnaire demonstrated similar results to a 24-hour dietary log administered by an interviewer (non-significant p-value) [6]. In another study on tooth brushing habits by young children evaluated through interviews, agreement was low between mothers’ responses conducted with a six-year interval [9]. In the present study, mothers may have reported better habits than their children customarily have. For instance, the mothers more frequently reported lower quantities on the item “amount of dentifrice dispersed on the brush”. However, the observed amount of dentifrice dispersed on the brush was nearly equally distributed. Moreover, a total of 81.1% of the mothers reported that an adult normally brushed the child’s teeth, whereas this figure was only 66.2% based on the examiner’s observations. These findings demonstrate a tendency toward reporting healthier habits.

This difference in the prevalence of habits depending on the method employed may be due to information bias. Some questions may also have confused the mothers as to how they behave and what they believe, which is a problem that can occur in epidemiological surveys [22]. For example, if a mother believes the correct amount of

dentifrice is ”less than ½ of the bristles”, she may mark this option even if it is not the actual behaviour. Moreover, mothers who have older children may confuse one child’s habits with those of another or consider all children’s habits equal. Currently, studies are carried out with busy mothers who work and may not be willing to spend much time answering a questionnaire. Moreover, a child may be under the care of grandparents or a baby sitter who may be more familiar with the child’s habits.

The observed method should also be evaluated with caution. On the item ”who disperses the dentifrice on the brush?”, a total of 81.1% of mothers reported that an adult dispersed the dentifrice on the brush, whereas the examiner observed this behaviour in 99.5% of the cases ($p < 0.001$). If one considers that bias is more frequent in reported information, more children would be expected to disperse the dentifrice on the brush themselves when observed by the examiner, which would be closer to reality. However, more mothers did so, indicating that they tended to behave differently in front of the researcher, possibly to appear that they are careful mothers. This item achieved the lowest Kappa agreement (0.00). Considering the formula given in the Methods section, K= po- pe/ 1- pe [11], the Kappa calculation for this item is expected to be pe = $[(38x1) + (163x200)/201^2 = 0.81$; K= 0.81 – 0.81/ 1- 0.81 = 0.0. This explains why this item achieved the lowest Kappa value, while overall agreement was the highest, as overall agreement considers the proportion of agreement by the total (po = 162/201 = 0.81) and not by chance (Kappa).

The present study has limitations that should be considered. There was only one observation of the tooth brushing. Video recordings of the tooth brushing could be an alternative to enable more reliable intra-examiner and inter-examiner comparisons [23]. Since the mothers and children knew they were being observed, they may have acted differently so as to appear that they are careful with oral hygiene, as stated above. Although they were not formally instructed and were left free to perform tooth brushing as they would at home, some incorrect behaviour may have been occurred. In the scholastic programme of the day care centres surveyed, there is only one meeting

with parents per semester. It was not possible to schedule other meeting in order to re-administer the questionnaire, which rendered a re-test assessment impossible. Although no intra-mother agreement test was performed, the sample was consistent for the investigation of agreement between mothers and observer. Finally, as the sample was comprised of young children, some of whom could still be learning or developing the habit (particularly for the items “the child spit the dentifrice out” and “the child rinsed the mouth”), many children may have swallowed the dentifrice because they were unable to spit it out correctly. However, the decision was made to conduct the study with young children because such children are the target population in studies on fluoride intake and the risk of dental fluorosis [4, 24, 25].

Both methods may have bias and the results should be interpreted with caution when conducting a study. The data collection process and goals of the study should be carefully and exhaustively explained to the participants and volunteers should be instructed to be sincere when participating in epidemiological studies so that the degree of bias, which is expected in epidemiological research, does not invalidate the study. Other strategies could help overcome this problem, such as the sample size, statistical analysis and the conduction of a pilot study prior the main evaluation. Moreover, neither mothers’ reports nor the observation method should be excluded from study designs. Researchers depend on these kinds of data and there is a genuine need for papers addressing reliability. Researchers should be aware of the study limitations and attempt not to influence the participants to adopt biased answers or behaviour. Additionally, the most adequate method should be chosen to fit the goal of the study and must be reliable, valid and cost effective for achieving the intended results, as the wrong choice of method can lead to questionable and conflicting results.

Conclusion

There was low agreement between observed brushing and mothers' reports. Moreover, the frequency of habits was different between the methods employed, suggesting that children's tooth brushing habits as reported by mothers and determined by observation data should be considered with caution in epidemiological surveys on fluoridated dentifrice use and the risk of dental fluorosis.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MJL participated in the study design and data acquisition. CCM participated in the study design, performed the statistical analysis, prepared the first draft and revised the manuscript. SMP and IAP conceived the study, developed the project design, protocols and revised the manuscript. All authors read and approved the final manuscript.

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Table 1: Comparison of mothers' reports and tooth brushing habits observed by examiner

Mothers' report		Observed by the examiner						
Kind of dentifrice child uses		**Children's**	**Adult's**		**Prevalence %**	**p-value†**	**Agreement %**	**K (SE*)**
	Children's	92	7		49.3	0.043	87.6	0.75 (0.05)
	Adult's	18	84		50.7			
	Prevalence %	54.7	45.3					
Amount of dentifrice dispensed on brush		**- ½ of bristles**	**½ of bristles**	**All bristles**	**Prevalence %**	**p-value†**	**Agreement %**	**K (SE*)**
	- ½ of bristles	47	28	8	41.9	<0.001	47.0	0.22 (0.05)
	½ of bristles	18	28	45	46.0			
	All bristles	2	4	18	12.1			
	Prevalence %	33.8	30.3	35.9				
Who dispenses dentifrice on toothbrush?		**Child alone**	**An adult**		**Prevalence %**	**p-value†**	**Agreement %**	**K (SE*)**
	Child alone	0	38		18.9	<0.001	80.6	0.00 (0.01)
	An adult	1	162		81.1			
	Prevalence %	0.5	99.5					
Who brushes child's teeth?		**Child alone**	**An adult**		**Prevalence %**	**p-value†**	**Agreement %**	**K (SE*)**
	Child alone	30	8		18.9	<0.001	77.1	0.43 (0.07)
	An adult	39	125		81.1			
	Prevalence %	33.8	66.2					
Does child spit out dentifrice during brushing?		**Yes**	**No**		**Prevalence %**	**p-value†**	**Agreement %**	**K (SE*)**
	Yes	67	11		39.6	<0.001	50.2	0.11 (0.05)
	No	87	32		60.4			
	Prevalence %	78.2	21.8					
Does child rinse out mouth during brushing?		**No**	**Yes**		**Prevalence %**	**p-value†**	**Agreement %**	**K (SE*)**
	No	10	3		6.5	<0.001	61.5	0.11 (0.04)
	Yes	74	113		93.5			
	Prevalence %	42.0	58.0					

*Standard error; † McNemar test

CONSIDERAÇÕES FINAIS

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Para as diferentes propostas de investigação desta pesquisa, as seguintes considerações finais podem ser feitas:

- Para o efeito da interrupção da ingestão de fluoretos (F) na água e nos dentifrícios sobre a excreção urinária em crianças de 2 a 4 anos de idade, observou-se um rápido decréscimo da excreção de F em 24 horas, partir do qual, a média de excreção urinária de fluoretos (EUF) se estabilizou com pequenas flutuações. No entanto, os níveis de excreção de F não voltaram aos níveis iniciais após o restabelecimento da ingestão de F a partir da água e do dentifrício. Estes resultados sugerem que como as crianças estão em alta taxa metabólica, e provavelmente o F ingerido foi principalmente retido pelos ossos. Além disso, é importante um controle e vigilância do teor de F na água dos municípios, bem como atenção para o uso contínuo de dentifrícios sem F por crianças. Tem havido um aumento do uso de dentifrícios sem F ou com baixas concentrações de F, que, no entanto, ainda não tiveram sua eficácia clínica comprovada cientificamente. Os achados poderiam ser diferentes se os teores de F na água de Ibiá tivessem se mantido constantes e/ou em níveis ótimos. Portanto, mais estudos sobre a farmacocinética do F no organismo são fundamentais para trazer informações sobre para a compreensão do mecanismo orgânico do fluoreto.
- Sobre a comparação entre os hábitos de escovação relatada pelas mães e a escovação observada pelo pesquisador, houve diferença estatisticamente significativa entre os dois métodos, sendo que os valores do coeficiente de Kappa variaram de ruim a moderado (0 a 0,43), exceto por uma questão (Tipo de dentifrício que a criança usa), cujo valor de Kappa foi bom (0,75). Os dois métodos podem apresentar

vieses se não forem corretamente administrados. É importante explicar aos voluntários sobre a importância de serem sinceros ao responder os questionários, de manterem o comportamento que eles adotam usualmente e de que não existem respostas “certas” ou “erradas”. Um estudo piloto pode ajudar a superar os problemas da coleta de dados. Também é fundamental que o pesquisador não adquira comportamentos que podem induzir tendências ou vieses. Finalmente, nenhum método deve ser excluído como instrumento de pesquisa. O ideal é escolher o método mais confiável para minimizar os vieses, de acordo com o assunto do estudo. Por exemplo, um estudo sobre uso de drogas por adolescentes ou doenças sexualmente transmissíveis, poderia ser mais bem aplicado através de questionários ao invés de entrevistas, uma vez que o primeiro método deixaria o voluntário livre para se expressar sem constrangimento. Para avaliação da dose de exposição ao F e risco de fluorose dental, o método da dieta duplicada e coleta dos resíduos da escovação permanecem sendo os métodos de escolha, apesar da possibilidade de viés.

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Considerações Iniciais

REFERÊNCIAS

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APÊNDICES

APÊNDICE 1

UFMG | **Universidade Federal de Minas Gerais**
Comitê de Ética em Pesquisa da UFMG - COEP

Parecer nº. ETIC 279/07

Interessado(a): Prof. Saul Martins de Paiva
Depto. Odontopediatria e Ortodontia
Faculdade de Odontologia -UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 27 de junho de 2007, o projeto de pesquisa intitulado **"Ingestão e excreção de flúor pela urina durante o uso de dentifrícios com diferentes concentrações de flúor"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Dra. Maria Elena de Lima Perez Garcia
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – Cep: 31270-901 – BH-MG
Telefone: (031) 3499-4592- FAX: (031)3499-4516 - e-mail: prpq@coep.ufmg.br

APÊNDICE 2

Ibiá, 25 de abril de 2008.

Instituto Educacional Crescendo para Vida

Por este instrumento, eu, Fabiana Borges, diretora e responsável pelo Instituto Educacional Crescendo para Vida , localizada à Rua 20, número 788, Ibiá, MG, declaro ter sido devidamente esclarecida pela cirurgiã-dentista Carolina de Castro Martins (fone: [redacted] doutoranda em Odontopediatria pela UFMG, que o objetivo da pesquisa "**Ingestão e excreção de flúor pela urina durante o uso de dentifrícios com diferentes concentrações de flúor",** é avaliar a concentração de flúor na urina e a exposição de crianças ao flúor.

Declaro, também, ter conhecimento que será necessária a participação de crianças na faixa etária entre 3 a 4 anos, alunos regularmente matriculados nesta escola.

Autorizo a realização da pesquisa na minha escola e com meus alunos, desde que cada família expresse sua vontade em participar através da assinatura do Termo de Consentimento Livre e Esclarecido a mim apresentado.

Fabiana Borges
Diretora do Instituto Educacional Crescendo para Vida

APÊNDICE 3

UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE ODONTOLOGIA
DEPARTAMENTO DE ODONTOLOGIA E ORTODONTIA
Comitê de Ética em Pesquisa da UFMG – TEL: (XX) XXXX XXXX
FAX: (XX) XXXX XXXX

Sobre a pesquisa

Olá! Meu nome é Carolina de Castro Martins, sou cirurgiã-dentista e pesquisadora pela Faculdade de Odontologia da UFMG. O objetivo da pesquisa que estamos realizando é entender sobre a quantidade de flúor que a criança está eliminando na urina.

O flúor é uma substância presente na pasta de dente para combater a cárie dentária. Mas se a criança engole muita pasta de dente com flúor, pode causar pequenas manchas brancas nos dentes, chamada fluorose dentária.

Pastas de dente

A pesquisa será feita por 34 dias corridos, em que a criança usará a pasta de dente Malvatri Kids baby®, sem flúor, que será fornecida por mim. Durante os primeiros 28 dias, **o seu filho não poderá usar outro tipo de pasta de dente, somente a Malvatri.** Nos dias restantes, seu filho voltará a usar a pasta de dente normal que ele está acostumado.

O seu filho deverá escovar os dentes 3 vezes por dia, nem mais, nem menos. Os horários das escovações devem ser: após o café da manhã, após o almoço e após o jantar. Seu filho deve escovar os dentes do jeito que ele que ele está acostumado.

Coleta da urina

A urina do seu filho será coletada **um dia antes dele começar a usar a pasta de dente Malvatri Kids baby®.** A partir daí, será coletada urina em 11 dias indicados por mim (1, 2, 4, 8, 12, 16, 20, 24, 32, 34). A coleta será feita por um período de **um**

dia completo, ou seja, **24 horas**. Toda urina de 24 horas deverá ser coletada. Não se preocupe, estarei com você sempre para acompanhá-la e lembrá-la dos dias da coleta. Os potes para coleta da urina serão fornecidos por mim, sem custo nenhum para você.

Durante a pesquisa:

- Durante o período da pesquisa, seu filho não poderá usar outra pasta de dente. **Somente a pasta de dente Malvatri Kids baby®.**
- Seu filho deverá escovar os dentes 3 vezes o dia, da forma que ele está acostumado.

Seu filho não deve fazer uso dos seguintes tipos de alimentos:

- chás (qualquer um deles);
- peixes ou frutos do mar;

Seu filho não deve fazer uso dos seguintes produtos bucais:

- gel com flúor;
- bochechos ou enxagüatórios bucais;

*Caso seja necessário visita ao dentista durante o período da pesquisa, favor me informar.

*Caso o Malvatri do seu filho acabe, favor me informar para que eu possa lhe dar outro.

Qualquer dúvida, favor entrar em contato comigo:

Carolina de Castro Martins

Fones: [redacted]
[redacted] (Ibiá)

APÊNDICE 4

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Por este instrumento, eu_____ , responsável pelo menor _____ de _____ anos de idade, declaro ter sido esclarecido (a) pela cirurgiã-dentista Carolina de Castro Martins (fone: ([redacted] - [redacted], que o objetivo da pesquisa é entender sobre a quantidade de flúor eliminada na urina. Afirmo saber que a pesquisa irá durar 34 dias; e que meu filho usará a pasta de dente Malvatri Kids baby® sem flúor por 28 dias corridos. Informo saber que esta pasta de dente não trará qualquer tipo de risco para o meu filho. Sei que a urina do meu filho será coletada um dia antes dele começar a usar a pasta Malvatri, e após iniciar o uso da pasta Malvatri, a urina será coletada em 11 dias alternados no decorrer do mês. A coleta da urina será feita durante um dia completo. As coletas não serão feitas de forma invasiva ou que tragam algum tipo de desconforto. Meu filho não será submetido a nenhum tipo de tratamento de dente. Fui esclarecido(a) que os dados deste estudo serão usados apenas pela equipe de pesquisadores, e utilizados para tese de doutorado em Odontologia pela pesquisadora, em que se tornarão públicos após a sua defesa. Minha identidade e a do meu filho não serão reveladas. Autorizo a minha participação e a participação de meu filho, o que mostra nosso interesse em colaborar com a pesquisa. É minha escolha participar ou não. Posso desistir em qualquer época, sem que eu seja prejudicado (a).

Em caso de dúvida, ligar para o COEP.

Ibiá, ____ de _____ de _____.

_____

Assinatura do responsável

APÊNDICE 5

Ficha da criança

Criança: _____

Nascimento: _____ Idade: _____

Mãe: _____profissão: _____

Pai: _____profissão: _____

Quem cuida: _____

Endereço:_____

_____

_____

Telefone:_____

Freqüenta escola: _____

Período: ( ) manhã ( ) tarde ( ) integral

Escova os dentes: ( ) sim ( ) não

Freqüência de escovação: _____

Qual pasta de dente usa: _____

Problemas de saúde: _____

Uso de medicamentos:_____

_____

Problemas bucais: _____

Usa fralda: ( ) dia inteiro ( ) noite ( ) não

_____

_____

Ingestão de líquidos: ( ) refrigerante ( ) chá ( ) leite em pó

( ) leite de caixa ( ) suco de caixa, garrafa ou natural

_____

_____

Água usada para beber: ( ) encanada ( ) mineral

Água usada para preparo dos alimentos: ( ) encanada ( ) mineral

Água usada para preparo do leite e suco: ( ) encanada ( ) mineral

APÊNDICE 6

Universidade Estadual de Campinas

Faculdade de Odontologia de Piracicaba

Departamento de Ciências Fisiológicas

Análise de Flúor

Solicitante: Carolina de Castro Martins

Endereço:

Cidade: Belo Horizonte - MG

Enviada: 12/05/07 **Recebida: 12/05/07**

Analisada: 14/05/07 **Remetida: 14/05/07**

código	AMOSTRAS	ppm F
1	Água do deionizador de Ibiá	<0,01

Piracicaba, 14 de Março de 2008.

Profa. Dra. Cínthia Pereira Machado Tabchoury
Área de Prestação de Serviços de Pequena Monta
FOP/Lab Bioquímica Oral/UNICAMP
Executora

PS: De acordo com a Portaria nº 635/BSB de 26/12/1975 do Ministério da Saúde, 0,7 mg F/L é a concentração ótima, sendo 0,6 e 0,8 mg F/L, considerados respectivamente como valores mínimo e máximo, para cidades com média das temperaturas máximas diárias de 26,8 a 32,5ºC.

Faculdade de Odontologia de Piracicaba
Av. Limeira, 901 - Caixa Postal 52
CEP 13414-903- Piracicaba - SP – Brasil
Telefone: [redacted] - Fax: [redacted]

Bioquímica
Telefone ([redacted] / [redacted]
E-mail:Home page: http://www.unicamp.br/fop

APÊNDICE 7

UFMG | **Universidade Federal de Minas Gerais**
Comitê de Ética em Pesquisa da UFMG - COEP

Parecer nº. ETIC 278/07

Interessado(a): Prof. Saul Martins de Paiva
Depto. Odontopediatria e Ortodontia
Faculdade de Odontologia -UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 27 de junho de 2007, o projeto de pesquisa intitulado **"Ingestão de flúor por meio do uso de dentifrícios com sabor regular e infantil: Estudo em pré-escolares"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Dra. Maria Elena de Lima Perez Garcia
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2° andar – Sala 2005 – Cep: 31270-901 – BH-MG
Telefone: (031) 3499-4592- FAX: (031)3499-4516 - e-mail: prpq@coep.ufmg.br

APÊNDICE 8

SERVIÇO PÚBLICO DO ESTADO DE MINAS GERAIS
SECRETARIA DE ESTADO DE EDUCAÇÃO
SUPERINTENDÊNCIA REGIONAL DE ENSINO DE MONTES CLAROS
GABINETE

A Cirurgiã-Dentista Maria José Lages de Oliveira e o Prof. Saul Martins de Paiva, respectivamente, aluna e professor do Programa de Pós-Graduação da Faculdade de Odontologia da Universidade Federal de Minas Gerais (Belo Horizonte), nível Doutorado, área de concentração Odontopediatria, vão desenvolver uma pesquisa intitulada **"INGESTÃO DE FLÚOR POR MEIO DO USO DE DENTIFRÍCIOS COM SABOR REGULAR E INFANTIL: ESTUDO EM PRÉ-ESCOLARES"** .

Os dados serão coletados através de questionários e através da observação do hábito rotineiro de escovação dos alunos.

Os resultados deste trabalho serão utilizados na tese de doutorado da pesquisadora, podendo ser divulgados e tornados públicos através de publicações cientificas, preservando-se, no entanto a identidade de todos os participantes.

Por meio desse documento, manifesto minha ciência e autorização quanto à realização do projeto junto às escolas e pré-escolas públicas do município de Montes Claros (Minas Gerais, Brasil).

Montes Claros, 22 de maio 2007.

Profa. Maria Salete de Souza Nether
Diretora da Superintendência Regional de Ensino de Montes Claros

Maria Salete de Souza Nether
Diretor II - 22ª SRE - Masp 266.894

Avenida Mestra Fininha, 1.642, Bairro Jardim São Luiz – CEP 39403-222 – Montes Claros – MG
Fone:(38)3212-2191 – Fax:(38)3212-2435 – E-mail:sre.mclaros.gab@educacao.mg.gov.br – pág. 1 de 1

APÊNDICE 9

DECLARAÇÃO

Por este instrumento, eu ....., Diretor (a) da escola ..... situada a Rua ..... nº ....., bairro....., cidade de Montes Claros (MG), declaro ter sido devidamente esclarecido pela cirurgiã-dentista MARIA JOSÉ LAGES DE OLIVEIRA (fones: [redacted]/ ([redacted], que os objetivos desta pesquisa são: avaliar os hábitos de higiene bucal, quanto ao uso de pastas de dente durante a escovação de alguns alunos na faixa etária de dezoito a trinta e seis meses, matriculados neste estabelecimento de ensino.

Afirmo ter conhecimento de que os dados serão coletados através de questionários e através da observação do hábito rotineiro de escovação dos alunos selecionados. E que os resultados deste trabalho serão utilizados na tese de doutorado da pesquisadora, podendo ser divulgados e tornados públicos através de publicações científicas, preservando-se a identidade de todos os participantes.

Declaro também, que a decisão em participar desta pesquisa reflete o interesse em contribuir.

Montes Claros, ... de ..... 200...

_____

ASSINATURA DA DIRETORIA E CARIMBO

Colegiado de Pós-graduação em Odontologia - Universidade Federal de Minas Gerais
Tel: (XX)XXXX XXXX Fax: (XX)XXXX XXXX
Comitê de Ética em Pesquisa da UFMF Tel: (XX)XXXX XXXX
Fax: (XX)XXXX XXXX

APÊNDICE 10

UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE ODONTOLOGIA
DEPARTAMENTO DE ODONTOPEDIATRIA E ORTODONTIA
Telefone do COEP: (XX) XXXX XXXX

Termo de Consentimento Livre e Esclarecido

Prezados Pais:

Estamos querendo fazer uma pesquisa na escola onde seu filho estuda, para avaliar os hábitos de higiene de boca e uso de pastas de dente durante a escovação.

As pastas dentais, quando usadas incorretamente, podem provocar manchas brancas nos dentes, chamadas de fluorose dentária.

Essas manchas não prejudicam em nada as crianças, mas precisamos saber mais como elas acontecem.

Para isso, precisamos que você responda a um questionário sobre os hábitos de vida de seu filho, principalmente o de escovação.

Qualquer dúvida pode ligar para a cirurgiã-dentista Maria José Lages de Oliveira, fones: [redacted] – [redacted].

Nada do que você responder será revelado. Você pode desistir de participar da pesquisa a qualquer momento, se qualquer tipo de prejuízos para você e seu filho.

Acreditamos que, com o fim deste trabalho, nós vamos poder conhecer mais sobre a fluorose e quem sabe no futuro poder controlar essas manchas e evitar que elas ocorram.

Se você concorda em participar, por favor, assine esta autorização em duas vias, onde uma via ficará com o pai ou responsável e outra via ficará com a pesquisadora.

Obrigada,

Maria José Lages de Oliveira (cirurgiã-dentista)

Eu, _____, após entender os objetivos e métodos desta pesquisa concordo em participar como voluntário (a) fornecendo as informações necessárias e permitindo que a pesquisadora acompanhe a escovação dos dentes do meu filho (a).

Montes Claros, _____, _____ de _____.

_____
Assinatura dos pais ou responsáveis pela criança

APÊNDICE 11

DADOS DA CRIANÇA

Voluntário Nº _____(preenchido pela Dentista)

Nome: _____

Gênero: _____ Data de Nascimento: ___/____/___

Idade: ____ anos____ meses (preenchido pela Dentista)

Peso: _____ kg (preenchido pela Dentista)

Nome da Mãe: _____

Nome do Pai: _____

Responsável pelas informações: _____

Endereço: _____

Telefone: _____

QUESTIONÁRIO

1. Seu filho (a) está fazendo uso de algum medicamento? Sim: ____ Não: ____
2. Qual? _____
3. Seu filho (a) possui alguma doença sistêmica? Sim: ____ Não :____
4. Qual? _____
5. Seu filho (a) já foi ao dentista? Sim: ____ Não: ____
6. Seu filho (a) já fez aplicação de fluoreto profissional? Sim: ____ Não :____
7. Seu filho (a) usa pasta de dente na escovação? Sim: ____ Não:____
8. Qual é tipo de pasta mais usada pelo seu filho (a)? ☐ Infantil ☐ Adulto
9. Qual marca de pasta mais usada pelo seu filho (a)? _____
10. Toda a família usa a mesma pasta dental? Sim: ____ Não: ____
11. Seu filho usa outra marca de pasta dental além da citada acima?

    Sim: ____ Não: ____
12. Caso você afirme que sim, qual é a outra marca de pasta que seu filho (a) usa além da citada acima?_____
13. Quando seu filho (a) começou a escovar os dentes com pasta dental?

    ☐ Antes de 1 ano de idade
    ☐ 1 ano de idade
    ☐ 2 anos de idade

14. Número de escovação feitas, em casa, por seu filho (a):

☐ Nenhuma vez

☐ 1 vez por dia

☐ 2 vezes por dia

☐ 3 vezes por dia

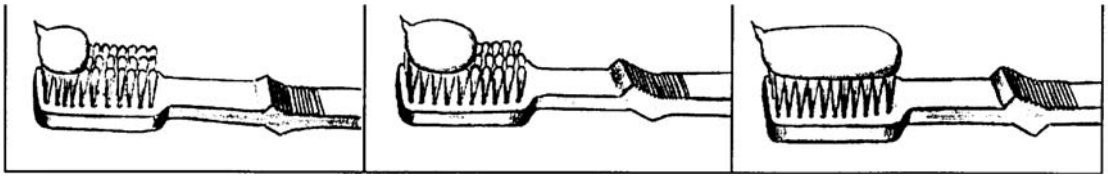
☐ 4 ou mais vezes por dia

15. Quem realiza a escovação dos dentes do seu filho (a)? _____

16. Quem coloca a pasta na escova do seu filho (a)? _____

17. Que tipo de escova é utilizado na escovação? Infantil_____ Adulto_____

18. Marque com um X quanto de pasta é colocado sobre a escova?



☐ Menos da metade das cerdas ☐ Metade das cerdas ☐ Toda extensão das cerdas

19. Seu filho (a) tem o hábito de escovar os dentes na escola?

Sim: ____ Não: ____

20. Número de escovação feitas, na escola, por seu filho (a):

☐ Nenhuma vez

☐ 1 vez por dia

☐ 2 vezes por dia

☐ 3 vezes por dia

☐ 4 ou mais vezes por dia

21. Seu filho (a) tem o hábito de engolir pasta dental na hora da escovação?

Sim: ____ Não: ____

22. Seu filho (a) tem o hábito de engolir pasta dental em outros momentos além da escovação? Sim: ____ Não: ____

23. Favor colocar um **X** nos itens que possuem em sua casa:

QUANTIDADES:

Item de posse		**Quantidades**			
	Não tem	**1**	**2**	**3**	**4 ou +**
Televisão em cores					
Rádio					
Banheiro					
Automóvel					
Empregada Mensalista					
Aspirador de pó					
Máquina de lavar					
Videocassete					
Geladeira					
Freezer (aparelho independente da geladeira duplex)					

24. Favor colocar um **X** no grau de instrução do chefe de família:

Analfabeto/ Primário incompleto	
Primário completo/ginasial incompleto	
Ginasial completo / colegial incompleto	
Colegial completo/superior incompleto	
Superior completo	

OBS.:_____

_____

Data: _____/_____/_____

APÊNDICE 12

International Journal of Environmental Research and Public Health

Instructions for Authors

Please first read the section 'Aims & Scopes' to have an overview, and to assess if your manuscript is suitable for this journal.

Submission

- Manuscript Preparation
- Potential Conflicts of Interest
- Review
- English Corrections
- Copyright / Open Access
- Reprints
- Supplementary Material

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- **Coverletter:** Check in your cover letter whether you supplied at least 5 referees. Check if the English corrections are done before submission.

Manuscript Preparation

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- **Reference Preparation:** References should preferably be prepared with EndNote ®, ReferenceManager ™or a similar bibliography software package. If references are prepared manually they must be checked for integrity and correctness (you may use ISI Web of Knowledge, PubMed/MEDLINE or Google Scholar). The Editorial Office will charge additional CHF 10 per citation for which extensive corrections must be made.
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writing a section) should be listed on the first page of the manuscript, below the title of the article. Other parties, who provided only minor contributions, should be listed under Acknowledgments only. A minor contribution might be a discussion with the author, reading through the draft of the manuscript, or performing English corrections.

- **Abstract and Keywords:** The abstract should be prepared as one paragraph (about 200 words). A list of three to ten keywords must be given, and placed after the Abstract.
- **Figures, Schemes and Tables:** Authors are encouraged to prepare figures and schemes in color. Full color graphics will be published free of charge. Figure and schemes must be numbered (Figure 1, Scheme I, Figure 2, Scheme II, *etc.*) and a explanatory title must be added. Tables should be inserted into the main text, and numbers and titles for all tables supplied. All table columns should have an explanatory heading. Please supply legends for all figures, schemes and tables. The legends should be prepared as a separate paragraph of the main text and placed in the main text before a table, a figure or a scheme.
- **Abstract/Table of Contents Graphic:** Authors are encouraged to provide a graphical representation of the paper (in either JPEG, GIF, PNG or PDF format) to be used as a graphic of the paper, along with the abstract, on the Table of Contents. The graphic should not exceed 500 pixels width/height. As an example, authors may review the abstract graphic of following papers: - http://www.mdpi.com/1424-8220/9/1/490 - http://www.mdpi.com/1420-3049/14/1/378
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Authors should suggest at least 5 potential referees with the appropriate technical expertise, although the Editor will not necessarily approach them. Please provide as detailed contact information as possible about the proposed referees (address, homepage address, phone and fax numbers and e-mail address). At least two of the suggested referees must be from a different country than the authors. Additionally, at least two suggested referees must be from a western country (USA, Canada, Japan, Australia or western European country). To identify potential referees, you may check the Editorial Board of our journal and suggest board members with appropriate research interests. In addition, we suggest searching for related papers in

Google Scholar and proposing their authors as possible reviewers. Another possibility is to propose authors that you frequently cited in your paper.

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- We wish to encourage the submission of supplementary data in electronic formats, so that important (scientific) information is retained in full. Electronic files or software regarding the full details of the calculation and experimental procedure, if unable to be published in a normal way, can be deposited as supplementary material
- Spectral data (NMR, IR, Raman, ESR, etc) can be submitted in JCAMP (.jdx) format. 3D coordinate structures (in pdb, mol, xyz or other common formats), if available, should also be submitted.

APÊNDICE 13

BMC Oral Health

Instructions for authors

The following word processor file formats are acceptable for the main manuscript document:

- Microsoft Word (version 2 and above)
- Rich text format (RTF)
- Portable document format (PDF)
- TeX/LaTeX (use BioMed Central's TeX template)
- DeVice Independent format (DVI)
- Publicon Document (NB)

Users of other word processing packages should save or convert their files to RTF before uploading. Many free tools are available which ease this process.

TeX/LaTeX users: We recommend using BioMed Central's TeX template and BibTeX stylefile. If you use this standard format, you can submit your manuscript in TeX format (after you submit your TEX file, you will be prompted to submit your BBL file). If you have used another template for your manuscript, or if you do not wish to use BibTeX, then please submit your manuscript as a DVI file. We do not recommend converting to RTF.

Note that figures must be submitted as separate image files, not as part of the submitted DOC/ PDF/TEX/DVI file.

Article types

When submitting your manuscript, you will be asked to assign one of the following types to your article:

Reserach article
Case report
Database
Debate
Software
Study protocol
Technical advance

Please read the descriptions of each of the article types, choose which is appropriate for your article and structure it accordingly. If in doubt, your manuscript should be classified as a Research article, the structure for which is described below.

Manuscript sections for Research articles

Manuscripts for Research articles submitted to *BMC Oral Health* should be divided into the following sections:

- Title page
- Abstract
- Background
- Methods
- Results
- Discussion
- Conclusions
- List of abbreviations used (if any)
- Competing interests
- Authors' contributions
- Authors' information (if any)

- Acknowledgements and Funding
- References
- Figure legends (if any)
- Tables and captions (if any)
- Description of additional data files (if any)

You can download a template (compatible with Mac and Windows Word 97/98/2000/2003/2007) for your article. For instructions on use, see below.

The **Accession Numbers** of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript should be provided, in square brackets and include the corresponding database name; for example, [EMBL:AB026295, EMBL:AC137000, DDBJ:AE000812, GenBank:U49845, PDB:1BFM, Swiss-Prot:Q96KQ7, PIR:S66116].

The databases for which we can provide direct links are: EMBL Nucleotide Sequence Database (EMBL), DNA Data Bank of Japan (DDBJ), GenBank at the NCBI (GenBank), Protein Data Bank (PDB), Protein Information Resource (PIR) and the Swiss-Prot Protein Database (Swiss-Prot).

Title page

This should list the title of the article. The title should include the study design, for example:

A versus B in the treatment of C: a randomized controlled trial

X is a risk factor for Y: a case control study

The full names, institutional addresses, and e-mail addresses for all authors must be included on the title page. The corresponding author should also be indicated.

Abstract

The abstract of the manuscript should not exceed 350 words and must be structured into separate sections: **Background**, the context and purpose of the study; **Methods**, how the study was performed and statistical tests used; **Results**, the main findings; **Conclusions**, brief summary and potential implications. Please minimize the use of abbreviations and do not cite references in the abstract; **Trial registration**, if your research article reports the results of a controlled health care intervention, please list your trial registry, along with the unique identifying number, e.g. **Trial registration**: Current Controlled Trials ISRCTN73824458. Please note that there should be no space between the letters and numbers of your trial registration number. We recommend manuscripts that report randomized controlled trials follow the CONSORT extension for abstracts.

Background

The background section should be written from the standpoint of researchers without specialist knowledge in that area and must clearly state - and, if helpful, illustrate - the background to the research and its aims. Reports of clinical research should, where appropriate, include a summary of a search of the literature to indicate why this study was necessary and what it aimed to contribute to the field. The section should end with a very brief statement of what is being reported in the article.

Methods

This should include the design of the study, the setting, the type of participants or materials involved, a clear description of all interventions and comparisons, and the type of analysis used, including a power calculation if appropriate.

Results and Discussion

The Results and Discussion may be combined into a single section or presented separately. Results of statistical analysis should include, where appropriate, relative and absolute risks or risk reductions, and confidence intervals. The results and discussion sections may also be broken into subsections with short, informative headings.

Conclusions

This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

List of abbreviations

If abbreviations are used in the text, either they should be defined in the text where first used, or a list of abbreviations can be provided, which should precede the competing interests and authors' contributions.

Competing interests

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Acknowledgements and Funding

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All references must be numbered consecutively, in square brackets, in the order in which they are cited in the text, followed by any in tables or legends. Reference citations should not appear in titles or headings. Each reference must have an individual reference number. Please avoid excessive referencing. If automatic numbering systems are used, the reference numbers must be finalized and the bibliography must be fully formatted before submission.

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Web links and URLs should be included in the reference list. They should be provided

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BMC Oral Health reference style

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- BibTeX
- EndNote style file
- Reference Manager
- Zotero

Article within a journal

1. Koonin EV, Altschul SF, Bork P: **BRCA1 protein products: functional motifs.** *Nat Genet* 1996, **13:**266-267.

Article within a journal supplement

2. Orengo CA, Bray JE, Hubbard T, LoConte L, Sillitoe I: **Analysis and assessment of ab initio three-dimensional prediction, secondary structure, and contacts prediction.** *Proteins* 1999, **43**(Suppl 3):149-170.

In press article

3. Kharitonov SA, Barnes PJ: **Clinical aspects of exhaled nitric oxide.** *Eur Respir J*, in press.

Published abstract

4. Zvaifler NJ, Burger JA, Marinova-Mutafchieva L, Taylor P, Maini RN: **Mesenchymal cells, stromal derived factor-1 and rheumatoid arthritis [abstract].** *Arthritis Rheum* 1999, **42:**s250.

Article within conference proceedings

5. Jones X: **Zeolites and synthetic mechanisms.** In *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Edited by Smith Y. Stoneham: Butterworth-Heinemann; 1996:16-27.

Book chapter, or article within a book

6. Schnepf E: **From prey via endosymbiont to plastids: comparative studies in dinoflagellates.** In *Origins of Plastids. Volume 2.* 2nd edition. Edited by Lewin RA. New York: Chapman and Hall; 1993:53-76.

Whole issue of journal

7. Ponder B, Johnston S, Chodosh L (Eds): **Innovative oncology.** In *Breast Cancer Res* 1998, **10:**1-72.

Whole conference proceedings

8. Smith Y (Ed): *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Stoneham: Butterworth-Heinemann; 1996.

Complete book

9. Margulis L: *Origin of Eukaryotic Cells.* New Haven: Yale University Press; 1970.

Monograph or book in a series

10. Hunninghake GW, Gadek JE: **The alveolar macrophage.** In *Cultured Human Cells and Tissues.* Edited by Harris TJR. New York: Academic Press; 1995:54-56. [Stoner G (Series Editor): *Methods and Perspectives in Cell Biology,* vol 1.]

Book with institutional author

11. Advisory Committee on Genetic Modification: *Annual Report.* London; 1999.

PhD thesis

12. Kohavi R: **Wrappers for performance enhancement and oblivious decision graphs.** *PhD thesis.* Stanford University, Computer Science Department; 1995.

Link / URL

13. **The Mouse Tumor Biology Database** [http://tumor.informatics.jax.org/mtbwi/index.do]

Link / URL with author(s)

14. Neylon, C: **Open Research Computation: an ordinary journal with extraordinary aims.** [http://blogs.openaccesscentral.com/blogs/bmcblog/entry/open_research_computation_an_ordinary]

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return to top

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- Description of data

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- Movies
- MOV (QuickTime)
- MPG (MPEG)
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4. Access the index.html file and browse around the mini-website, to ensure that the most commonly used browsers (Internet Explorer and Firefox) are able to view all parts of the mini-website without problems, it is ideal to check this on a different machine
5. Compress the folder into a ZIP, check the file size is under 20 MB, ensure that index.html is in the root of the ZIP, and that the file has .zip extension, then submit as an additional file with your article

Style and language

General

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Gene names should be in italic, but protein products should be in plain type.

There is no explicit limit on the length of articles submitted, but authors are encouraged to be concise. There is no restriction on the number of figures, tables or additional files that can be included with each article online. Figures and tables should be sequentially referenced. Authors should include all relevant supporting data with each article.

BMC Oral Health will not edit submitted manuscripts for style or language; reviewers may advise rejection of a manuscript if it is compromised by grammatical errors. Authors are advised to write clearly and simply, and to have their article checked by colleagues before submission. In-house copyediting will be minimal. Non-native speakers of English may choose to make use of a copyediting service.

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The abstract is one of the most important parts of a manuscript. For guidance, please visit our page on "Writing titles and abstracts for scientific articles" Tim Albert has produced for BioMed Central a list of tips for writing a scientific manuscript. MedBioWorld also provides a list of resources for science writing.

Abbreviations

Abbreviations should be used as sparingly as possible. They can be defined when first used or a list of abbreviations can be provided preceding the acknowledgements and references.

Typography

Please use double line spacing.

- Type the text unjustified, without hyphenating words at line breaks.
- Use hard returns only to end headings and paragraphs, not to rearrange lines.
- Capitalize only the first word, and proper nouns, in the title.
- All pages should be numbered.
- Use the *BMC Oral Health* reference format.
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  Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF.
- Genes, mutations, genotypes, and alleles should be indicated in italics, and authors are required to use approved gene symbols, names, and formatting. Protein products should be in plain type.

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Resumos de trabalhos científicos apresentados e publicados em anais de eventos científicos (*apresentador)

Martins CC*, Ramos-Jorge ML, Paiva SM, Cury JA. Viés de memória: reprodutibilidade de entrevista realizada com a mesma população em um tempo baseline e 6 anos depois. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE PESQUISAS ODONTOLÓGICAS, 20, 2006, Atibaia. **Anais...** São Paulo: SBPqO, set. 2006. p. 289. (Resumo Pc 181).

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