GIOVANA DANIELA PECHARKI

ANÁLISE MULTINÍVEL DE FATORES SOCIAIS E BIOLÓGICOS ASSOCIADOS À CÁRIE DENTÁRIA

CURITIBA

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde (PPGCS) do Centro de Ciências Biológicas e da Saúde (CCBS) da Pontifícia Universidade Católica do Paraná (PUCPR), como parte dos requisitos para a obtenção do título de Doutor em Ciências da Saúde, Área de Concentração Medicina.

Orientadora: Profa. Dra. Paula Cristina Trevilatto

Co-Orientador: Prof. Dr. Samuel Jorge Moysés

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DEDICATÓRIA

Dedico às minhas sobrinhas fascinantes: **Julia e Victoria**, que são as melhores "*teses"* já realizadas no mundo e que comprovam a *"hipótese"* de que a vida, para ser plena, tem que ser *leve* (como o dançar da Julia) e ao mesmo tempo *profunda* (como o olhar da Victoria). Pessoinhas que aumentaram extraordinariamente o *"Fator de Impacto"* positivo da minha vida e deram-na muito mais *"Qualis".*

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“Não existem dias feios,

todos os dias são bonitos,

uns são claros,

outros são escuros,

outros são chuvosos,

Mas todos os dias são bonitos...”

JULIA PECHARKI MARQUES DA SILVA (SOBRINHA)

No momento com apenas quatro anos de idade, mas muitos anos de sabedoria...

Resumo

RESUMO

A cárie dentária é uma doença complexa que apresenta natureza multifatorial. Assim, uma abordagem considerando fatores que podem influenciar a doença em diferentes níveis e a sua integração parece ser desejável. A Análise Multinível (AM) é um modelo que considera variáveis envolvidas no desfecho da doença desde o nível individual até o nível populacional. O objetivo deste estudo foi investigar, por meio de AM, a associação de fatores sociais e biológicos, agrupados em níveis hierárquicos, com a cárie dentária em estudantes de 12 anos de idade. O estudo avaliou 687 estudantes, de ambos os sexos, de seis escolas públicas e seis escolas privadas em seis diferentes distritos sanitários de Curitiba, Brasil. Foram avaliados os seguintes parâmetros: i) *nível individual*- sexo, etnia, posição socioeconômica, hábitos em saúde bucal (escovação, uso de fio dental, uso de fluoreto tópico, dieta, acesso público ou privado e freqüência de visitas ao dentista), acúmulo de biofilme dental, gengivite, fluorose e parâmetros salivares (fluxo e capacidade tampão); ii) *nível escolar*- tipo de escola (pública ou privada), educação em saúde bucal, permissão para consumo de doces, e iii) *nível distrital*- concentração de flúor na água de abastecimento e condições socioeconômicas. O *software* MLwiN foi utilizado para estimar os efeitos das variáveis do indivíduo, escola e distrito sobre a experiência de cárie. Os principais achados foram que variáveis individuais apresentaram uma associação significativa com a experiência de cárie, mesmo na presença dos níveis escolar e distrital (variáveis contextuais). Verificou-se que o sexo masculino esteve associado negativamente com a experiência de cárie. No entanto, a interação entre sexo masculino e não uso de fluoreto tópico foi positivamente associada com a cárie dentária. Posições socioeconômicas menores, acúmulo de biofilme e fluorose foram significativamente associados com a experiência de cárie. Entretanto, a interação entre acúmulo de biofilme e fluorose foi negativamente associada com o desfecho de cárie. Além disso, a interação entre o não uso de fio dental e a utilização de serviços odontológicos públicos foi associada com a experiência de cárie. Os fatores individuais estiveram associados com a experiência de cárie mesmo com a inclusão de variáveis contextuais na população estudada.

Abstract

ABSTRACT

Dental caries is a complex disease which has a multifactorial nature. Thus, an approach, considering factors that may influence disease at different levels and their integration, is desirable. Multilevel Modelling (MM) is a clustered analysis that considers variables involved in the disease outcome from the individual to the populational level. The aim of this study is to investigate, through MM, the combination of social and biological factors, grouped into hierarchical levels, with dental caries in 12 year-old students. The study evaluated 687 students, both sexes, from six public and six private schools from six different health districts of Curitiba, Brazil. The following parameters were evaluated: i) individual level: sex, ethnic group, socioeconomic status, oral health behavior (toothbrushing, flossing, fluoride use, diet, dental services access, frequency of dental visits), dental biofilm accumulation, gingivitis, fluorosis, salivary parameters (flow rate, buffer capacity); ii) school level: type of school (public or private), oral health education, permission for sweeties consumption, and iii) district level: concentration of fluoride in water supply, and district socioeconomic conditions. The multilevel software MLwiN was used to estimate the effects of individual, school and district variables on caries experience. The main findings were that individual variables had a highly significant association with caries experience even in the presence of school and district levels (contextual variables). It was found that male sex negatively associated with caries experience. However, the interaction between male sex and no fluoride use was positively associated with caries experience. Lower socieconomic status, dental plaque accumulation, and fluorosis were significantly associated with caries experience. Nevertheless, the interaction between dental biofilm accumulation and fluorosis was negatively associated with caries experience. In addition, the interaction between no flossing and use of public dental services were associated with caries outcome. It was observed that individual factors were associated with caries experience even after the inclusion of contextual variables in the study population.

Sumário

SUMÁRIO

Introdução

1 INTRODUÇÃO

1.1 CONCEITO E MECANISMO DA DOENÇA CÁRIE

A cárie dentária é de natureza infecciosa e pertence ao grupo das doenças complexas ou multifatoriais, tais como o câncer, doenças cardíacas e o diabetes (Fejerskov, 2004). É resultante do desequilíbrio entre os processos de desmineralização e remineralização do esmalte dentário (Fejerskov, 1998; Featherstone, 2004), e não representa um processo único, mas uma sucessão de eventos em determinado tempo (Newbrun, 1978). As lesões cariosas podem surgir quando microrganismos cariogênicos (van Houte, 1994; Adji et el., 2002) e carboidratos fermentáveis (Burt & Pai, 2001) estão presentes em um hospedeiro suscetível (Singh et al., 2002). Na doença, ocorre a destruição de sítios de tecido dentário por meio da fermentação de carboidratos da dieta em subprodutos ácidos a partir de bactérias (Marsh, 2006). Os sinais da desmineralização são observados sobre os tecidos mineralizados do dente; no entanto, o processo da doença já se inicia no local do biofilme dental (placa bacteriana), que recobre a superfície dentária (Selwitz et al., 2007). As lesões cariosas podem se desenvolver onde a maturidade e permanência de biofilme sobre os dentes ocorrem por longos períodos. A cavidade fornece um nicho ecológico, no qual microrganismos do biofilme dental gradualmente irão se adaptar a um pH reduzido (Fejerskov, 2004). A formação de uma lesão cavitada parece proteger a comunidade bacteriana e o processo carioso pode evoluir, caso não haja eliminação do biofilme (Kidd & Fejerskov, 2004).

A cárie dentária é uma das enfermidades preveníveis mais comuns, e a suscetibilidade à doença permanece ao longo da vida (Pitts, 2004; Fejerskov & Kidd, 2005). É considerada a principal causa de dor em ambiente bucal e de perda dentária (Kidd, 2000). Além disso, é reversível na sua fase inicial e pode ser interrompida a qualquer tempo, mesmo quando a dentina ou o esmalte são destruídos. É uma doença crônica que progride lentamente na maior parte dos indivíduos. Na prática diária, cirurgiões-dentistas, outros prestadores de serviços em saúde bucal e pacientes muitas vezes se referem à cárie como sendo uma cavidade no dente; no entanto, esta é considerada apenas a seqüela da doença, um sinal de avanço do processo carioso (Thylstrup & Fejerskov, 2000). A cárie pode apresentar

aumento da severidade e destruição que varia do nível sub-clínico, com mudanças ao nível molecular, até lesões cavitadas (Pitts, 2004b, Featherstone, 2004).

1.2 EPIDEMIOLOGIA DA CÁRIE DENTÁRIA

Estudos têm demonstrado uma consistente redução da prevalência da cárie dentária nos países desenvolvidos nas últimas décadas (Marthaeler et al., 1996). Esse fato tem sido também observado na América Latina e no Brasil (Narvai et al., 1999; Bonecker & Cleaton-Jones, 2003). Levantamentos epidemiológicos realizados no Brasil, com resultados válidos para a população amostral investigada, demonstram que, entre 1980 e 2003, o declínio nos valores do índice de dentes cariados, perdidos e restaurados (CPOD) em indivíduos de 12 anos foi de 61,7% (Narvai et al., 2006). O último levantamento epidemiológico realizado em nível nacional, ocorrido em 2002 e 2003, revelou um índice CPOD médio aos 12 anos de idade de 2,78 (Brasil, 2004), atingindo as metas estabelecidas pela Organização Mundial de Saúde (OMS) para o ano 2000 somente para essa idade (CPOD menor que 3,0) e, em parte, isso ocorreu devido aos menores índices encontrados nos adolescentes das regiões Sul e Sudeste. As causas mais prováveis para essa queda estão associadas ao uso generalizado de fluoretos, acesso aos serviços odontológicos, mudanças de critérios de diagnóstico de cárie e ampliação das ações de promoção e educação em saúde bucal (Nadanovsky & Sheiham,1994; Splieth et al., 2004; Narvai et al., 2006).

A idade de 12 anos é recomendada pela OMS como a idade de monitoramento global da cárie para comparações internacionais e o acompanhamento das tendências da doença, devido à facilidade de obtenção de amostra por meio do sistema escolar e porque é provável que nesta idade todos os dentes permanentes, com exceção dos terceiros molares, já tenham irrompido (OMS, 1997).

Embora os indivíduos de 12 anos apresentem valores de ataque de cárie semelhantes aos padrões recomendados pela OMS para o ano 2000 no Brasil, a variabilidade da distribuição dos valores é ampla e há uma proporção significativa de adolescentes nessa idade com valores muito elevados de ataque (Brasil, 2004).

Estima-se que 20 a 25% das crianças e adolescentes nos Estados Unidos e no Brasil concentram 60 a 80% da prevalência de cárie (Kaste et al., 1996; Narvai et al., 2006). Em outro estudo brasileiro, 17% das crianças apresentaram 46% de toda a cárie da população avaliada (Mattos-Graner et al., 1996). Este fenômeno de concentração da cárie em pequenos grupos é denominado *polarização* e representa um dos aspectos epidemiológicos da doença, na qual uma parcela da população concentra grande parte das necessidades de tratamento (Antunes et al., 2002; Narvai et al., 2006). Esses dados demonstram a necessidade da identificação precoce de indivíduos de maior risco à cárie, para que sejam adotadas medidas necessárias para a sua prevenção, controle e redução de danos, com óbvias conseqüências epidemiológicas, humanas e econômicas (Selwitz et al., 2007).

1.3 FATORES QUE PREDISPÕEM À CÁRIE DENTÁRIA

O risco individual à cárie inclui fatores socioeconômicos, comportamentais e biológicos (Selwitz et al., 2007). As variáveis que influenciam a susceptibilidade individual à doença são: a classe socioeconômica (Peres et al., 2007), hábitos em saúde bucal (Stecksen-Blicks et al., 2004) e aspectos biológicos, tais como acúmulo de biofilme e propriedades da saliva (Selwitz, et al., 2007).

A posição socioeconômica dos indivíduos foi significativamente associada com o índice CPOD (Antunes et al., 2004; Pattussi et al., 2006; Peres, et al., 2007). Evidências mostraram que o grau mais elevado da doença está diretamente relacionado às pessoas que apresentam menor renda (Baldani et al., 2002; Antunes et al., 2004). Além disso, os indivíduos com menor número de lesões cariosas foram aqueles cujos pais tinham um maior nível de educação (Peres et al., 2005; Pereira et al., 2007; Yazdani et al., 2008).

Em relação ao comportamento, a escovação é a mais conhecida de todas as práticas de higiene bucal; além disso, o uso regular de fio dental é universalmente recomendado (Peres et al. 2007; Honkala et al., 2007; Aida et al., 2008; Ayo-Yusuf et al., 2008). A utilização de dentifrício e outras fontes de fluoreto tópico também pode ser efetivamente orientada para a prevenção de cárie (Levin et al., 2008; Aida et al., 2008; Marinho, 2008). O padrão de utilização de serviços odontológicos, que

envolve o acesso público ou privado e a freqüência de visitas ao cirurgião-dentista, também tem sido associado com a saúde bucal (Peres et al. 2007, Weyant et al., 2007). Adicionalmente, a freqüência elevada de consumo de açúcar foi positivamente relacionada à cárie dentária (Pattussi et al., 2006; Aida et al., 2008). Além disso, associações de aspectos comportamentais com a condição socioeconômica e a saúde bucal têm sido relatadas (Peres et al., 2000; Stecksen-Blicks et al., 2004), permitindo sugerir que quanto maior o nível socioeconômico, maior é a condição de controle da cárie (hábitos de higiene bucal, alimentares, acesso ao cuidado profissional).

A composição e a secreção da saliva também podem influenciar o ambiente intrabucal, protegendo os tecidos dentários (Lenander-Lumikari & Loimaranta, 2000; van Nieuw Amerongen et al., 2004). Um fluxo constante de saliva elimina eficazmente microrganismos da cavidade bucal e um fluxo reduzido (hipossalivação) leva a uma proliferação de bactérias, seguida de inflamação gengival (Atkinson & Baum, 2001; Tenovuo, 2002). Além disso, a saliva apresenta um sistema de tamponamento para a rápida neutralização de ácidos, que pode desmineralizar o esmalte e dentina (Lenander-Lumikari & Loimaranta, 2000; Leone & Oppenheim, 2001).

No entanto, a importância crescente de investigação das doenças crônicas em muitos países tem sido acompanhada por uma mudança de fatores de risco do nível individual para o nível populacional (Diez-Roux et al., 2003). Os fatores individuais geralmente podem ser influenciados pelo contexto populacional em relação à saúde geral e bucal (Holst et al., 2001; Diez-Roux et al., 2003; Aida et al., 2008).

O contexto populacional, bem como as características individuais, podem afetar a saúde bucal. No entanto, a contribuição de aspectos populacionais para a cárie dentária entre indivíduos de várias regiões permanece incerta quando somente a saúde individual é considerada (Aida, et al., 2008). Um maior CPOD foi observado em crianças que freqüentam escolas públicas (Antunes et al., 2006). As características da área geográfica também podem desempenhar um importante papel na explicação das desigualdades na cárie dentária (Antunes et al., 2004). Altos índices de CPOD foram significativamente menores em áreas com maiores níveis de controle social (Patussi et al., 2006). Foi observado um aumento

expressivo na severidade da cárie em bairros que apresentavam fatores econômicos desfavoráveis (Tellez et al., 2006). Também, características do bairro foram associadas com a autopercepção em saúde bucal (Turell et al., 2007). Além disso, a fluoração da água de abastecimento público tem demonstrado ser eficaz na redução da cárie em muitas comunidades (Antunes et al., 2006, Levin et al., 2008).

A possibilidade de conhecer fatores de risco para a cárie dentária em diferentes populações pode permitir adequar os cuidados de saúde bucal e re-orientar gastos em prevenção, respeitando-se assim o princípio da eqüidade. Adicionalmente, essa informação é útil para o planejamento estratégico e para o estabelecimento de diretrizes para ações de saúde bucal em sistemas locais de saúde, contribuindo, assim, para a eqüidade em saúde bucal (de Campos Mello et al., 2008).

1.4 ANÁLISE MULTINÍVEL DA CÁRIE DENTÁRIA

Em virtude da natureza multifatorial da cárie dentária, é desejável uma abordagem que considere fatores que possam influenciar a doença em diferentes níveis, e que vise a sua integração. A Análise Multinível (AM), consiste em um modelo que considera desde o nível individual da doença até níveis populacionais (Goldstein, 2003). A AM possibilita compreender os comportamentos individuais e seus efeitos, analisando não apenas as características dos indivíduos, mas também dos grupos sociais a que eles pertencem (Diez-Roux, 1998).

A AM permite a avaliação de variáveis de diferentes níveis hierárquicos, bem como suas interações, o que possibilita a análise global dos fatores que contribuem para o processo de cárie (minimizando a perda de poder estatístico, bem como o risco de falácia ecológica, um tipo de erro observado quando apenas o nível populacional é considerado). A AM permite estimar a variação que ocorre em níveis individuais e da comunidade (Aida et al., 2008) e proporcionam um meio eficiente de ligação entre distintos estudos convencionalmente ecológicos e os de nível individual (Tellez et al. 2006).

Para a realização da AM, geralmente se utiliza a regressão logística multivariada multinível, que leva em conta a estrutura hierárquica dos dados,

permitindo ajustar os resultados quanto a variáveis de confusão e considerando possíveis interações (Diez-Roux, 1998, Goldstein, 2003). Ao ignorar a estrutura hierárquica dos dados, como nos modelos de regressão tradicionais, estes são agregados em um único nível. As análises de dados agregados ou desagregados em um único nível introduzem problemas estatísticos e conceituais. Modelos multiníveis permitem não só calcular a contribuição de cada nível da hierarquia na magnitude da variação do desfecho de interesse, mas também fornecer estimativas dos efeitos de características das unidades de cada nível no desfecho (Cruz & Leon, 2005).

De acordo com Hox (1995), a análise multinível trabalha com o conceito de que os indivíduos interagem com os contextos sociais nos quais estão inseridos, ou seja, os indivíduos são influenciados pelos grupos sociais aos quais pertencem e que as características desses grupos, por outro lado, são influenciadas pelos indivíduos que deles fazem parte. Dessa forma, os indivíduos e os grupos são conceituados como um sistema hierárquico com indivíduos e grupos definidos em níveis separados.

Segundo Diez-Roux (1998), o principal aspecto diferencial da análise multinível com os outros modelos é que ele trabalha com uma teoria de causalidade que integra variáveis em níveis micro e macro, explicando a relação e interação entre os níveis. Mendonça (2001) ao discutir a análise multinível, aponta que as variáveis de grupo afetam os indivíduos diretamente ou interferem nas escolhas feitas por cada um.

Os modelos multiníveis podem ser utilizados em ensaios clínicos randomizados, estudos observacionais transversais e avaliações em serviços de saúde (Bingenheimer & Raudenbush, 2004).

As limitações relacionadas com o uso dos modelos multinível são: i) a dificuldade na identificação adequada dos níveis e suas variáveis, ii) conhecimento dos números apropriados de unidades de cada nível na amostra (Diez-Roux et al., 1998).

Apesar das dificuldades, essa metodologia estatística tem trazido maior capacidade de análise no âmbito da pesquisa empírica epidemiológica, e tudo indica que se tornará uma ferramenta padrão para os pesquisadores da área da saúde bucal (Cruz & Leon, 2005).

Proposição

2 PROPOSIÇÃO

O objetivo deste estudo foi descrever a prevalência de cárie na população estudada e analisar a associação das variáveis individuais e populacionais, agrupadas nos níveis hierárquicos individual, escolar e distrital, com a cárie dentária, em escolares de 12 anos de idade, por meio da Análise Multinível.

Artigo

3 ARTIGO

Multilevel Modelling of social and biological factors in dental caries

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Abstract

Background: Dental caries is a complex disease which has a multifactorial nature. Thus, an approach, considering factors that may influence disease at different levels and their integration, is desirable. Multilevel Modelling (MM) is a clustered analysis that considers variables involved in the disease outcome from the individual to the populational level. The aim of this study is to investigate, through MM, the combination of social and biological factors, grouped into hierarchical levels, with dental caries in 12 year-old students. **Methods:** The study evaluated 687 students, both sexes, from six public and six private schools from six different health districts of Curitiba, Brazil. The following parameters were evaluated: i) *individual level*: sex, ethnic group, socioeconomic status, oral health behavior (toothbrushing, flossing, fluoride use, diet, dental services access, frequency of dental visits), dental biofilm accumulation (plaque index), gingivitis, fluorosis, salivary parameters (flow rate, buffer capacity); ii) *school level*: type of school (public or private), oral health education, permission for sweeties consumption, and iii) *district level*: concentration of fluoride in water supply, and district socioeconomic conditions. The multilevel software MLwiN version 2.01 (Centre for Multilevel Modelling, Bristol, UK) was used to estimate the effects of individual, school and district variables on caries experience. **Results:** the main findings were that individual variables had a highly significant association with caries experience even in the presence of school and district levels (contextual variables). It was found that male sex negatively associated with caries experience. However, the interaction between male sex and no fluoride use was positively associated with caries experience. Lower socieconomic status, dental plaque accumulation, and fluorosis were significantly associated with caries experience. Nevertheless, the interaction between dental biofilm accumulation and fluorosis was negatively associated with caries experience. In addition, the interaction between no flossing and use of public dental services were associated with caries outcome. **Conclusion:** It was observed that individual factors were associated with caries experience even after the inclusion of contextual variables in the study population.

Introduction

Dental caries is an infectious condition that belongs to a complex group of multifactorial diseases, such as cancer, cardiovascular disorders and diabetes (Fejerskov, 2004). The prevalence of the disease has reduced significantly, including Latin America and Brazil (Marthaler et al., 1996; Narvai et al., 1999; Bonecker & Cleaton-Jones, 2003). Some authors argue that this reduction is possibly due to the increased availability of fluoride and the action of oral health programmes (Krasse, 1996; Splieth et al., 2004; Narvai et al., 2006).

Nevertheless, groups of children have still been showing high caries activity. It is estimated that 20 to 25% of children and adolescents in USA and Brazil concentrate 60 to 80% of the caries prevalence (Kaste et al., 1996; Narvai et al., 2006). In another Brazilian sample, 17% of children showed 46% of the whole caries lesions from the evaluated population (Mattos-Graner et al., 1996). This phenomenon of dental caries concentration in small groups is termed *polarization*, and represents one of the epidemiological disease aspects, in which a portion of the population has focused most needs for treatment (Antunes et al., 2002; Narvai et al., 2006).

These data demonstrate the importance of early identification of caries risk factors, for adopting adequate measures for its prevention, control and reduction of damage, with obvious epidemiological, human and economical consequences (Selwitz, et al., 2007).

The main risk factors influencing individual susceptibility to caries development could be considered the socioeconomic status (Peres et al., 2007), oral health behavior (Stecksen-Blicks et al., 2004), gender and ethnicity (Antunes et al., 2006, Pattussi et al., 2007) and also biological factors, such as biofilm formation and saliva proprieties (Selwitz, et al., 2007).

Socioeconomic position of individuals was significantly associated with decayed, missing or filled teeth (DMFT) index (Antunes et al., 2004; Pattussi et al., 2006; Peres, et al., 2007). Evidences show that the higher degree of disease outcome is directly related to lower income people (Baldani, 2002; Antunes et al., 2004). Moreover, subjects with caries were fewer among those whose parents had a high level of education (Pereira et al., 2007; Yazdani et al., 2008).

In relation to behavior toothbrushing is one of the most relevant of all oral hygiene practices; in addition, regular dental flossing is universally recommended (Peres et al. 2007; Honkala et al., 2007; Aida et al., 2008; Ayo-Yusuf et al., 2008).

Use of dentifrice and other sources of topical fluoride can be also effectively targeted to caries prevention (Levin et al., 2009; Aida et al., 2008; Marinho, 2008). The pattern of dental services use, which involves public or private access and frequency of dental visits, were also associated with oral health (Peres et al., 2007, Weyant et al., 2007). High frequency of sugar consumption was also positively linked to dental caries (Pattussi et al., 2006; Aida et al., 2008).

From the biological point of view, a high exposure to fermentable carbohydrates can modify the composition of the biofilm (Paes Leme et al., 2006), mainly represented by mutans and lactobacilli, favoring its cariogenicity (van Houte, 1994). Also salivary flow, buffering capacity and composition of saliva may influence the pathogenicity of the biofilm (Lenander-Lumikari & Loimaranta, 2000).

However, the growing importance of chronic diseases in many countries has been accompanied by a shift from individual to population–level factors in risk factor research (Diez Roux et al., 2003). Individual factors are usually influenced by the population context and its effects on general and oral health (Diez Roux et al., 2003; Holst et al., 2001; Aida et al., 2008). A higher DMFT and a lower dental care index were observed in children attending public schools than for those enrolled in private schools (Antunes et al., 2006). Geographical area characteristics may also play an important role in explaining inequalities in dental caries (Antunes et al., 2004). High DMFT rates were significantly lower in areas with higher levels of empowerment (Patussi et al., 2006). There is significant variation in the severity of caries between low-income neighborhood clusters (Tellez et al., 2006). Indeed, neighborhood features were seen to be associated with self-reported oral health (Turell et al., 2007). Moreover, fluoridation of public water supplies has proved to be successful in reducing caries in many communities worldwide (Antunes et al., 2006; Levin et al., 2009).

Because of the multifactorial nature of dental caries, an approach, considering factors that may influence the disease at different levels, and their integration, is desirable. Multilevel Analysis is a model which considers from individual to populational levels of disease (Goldstein, 2003).

The aim of this study was to investigate the association of individual and populational variables, grouped into hierarchical levels, with dental caries in 12-year-old students by Multilevel Modelling.

Methods

Curitiba city, Paraná State, Brazil, is a metropolis of 3 million people, composed of 8 health districts, from which 6 were selected for study. In each district, 2 schools were randomly chosen, one public and one private, totalizing 12 schools (fig 1). The sample was composed of 687 individuals, approximately 55 students from each school, 12 years old, both sexes. Adolescents were not included when smokers, using orthodontic appliances, and taking chronic anti-inflammatory and antibiotics in the last three months. Two individuals were excluded because they were diabetic. The students were allocated to the study only if the parent/caregiver returned the informed consent form, according to norms of the Ethical Committee on Research of the Center for Health and Biological Sciences of Pontifical Catholic University of Paraná (PUCPR), according to Resolution 96/96 of the Health National Council, register n°487.

A multilevel study was designed to assess the individual and population effects on caries experience (DMFT=0 or DMFT>0). The students were diagnosed according to the decayed, missing and filled teeth index (DMFT). Teeth were considered decayed when presenting either cavities or white lesions.

Data were hierarchically structured in three levels: students/individuals (level 1), schools (level 2), and districts (level 3).

Individual level (level 1)

Examinations were conducted in schoolrooms in accordance with international standards established by WHO. Plane mouth mirrors and community periodontal index (CPI) probes were used for conducting the examinations (WHO, 1997). All evaluations were performed by two calibrated examiners. Inter- and intra-examiner reproducibility was taken on 10% of the sample and the Kappa test was used to measure reliability. The obtained values for Kappa test were 0.93 for inter- and 0.99 for intra-examiner.

Individual demographic variables included sex (male/female) and skin colour/ethnicity: white, light- and dark-skinned black, and yellow.

The parent/caregiver answered self-completed questionnaires and any difficulty in understanding was checked. Oral health behavior questions asked about toothbrushing frequency, dental flossing, use of other sources of topical fluoride (solution, varnish, gel), dental attendance pattern (frequency of dental check-ups and

public/private access) and sugar consumption between meals (Pattussi et al., 2006; Peres, et al., 2007; Aida et al., 2008).

A standard Brazilian socioeconomic classification based on household items and on the level of education of the head of household was adopted (ABEP, 2008). This comprises a group of specific indicators such as number of bathrooms, number of full-time domestic servants, number of cars owned by the family, possession of domestic items such as television sets, radio sets, video cassette recorders, vacuum cleaners washing machine, fridges, freezers; and level of education of the head of family. A set of points is assigned to these indicators and a final score defines the socioeconomic groups; A (highest), B, C, D, and E (lowest). Because of the small number of observations in class E, data were categorized into four groups: high social class (class A), high-middle social class (class B), middle social class (classes C) and low social class (classes D and E).

The biofilm accumulation was verified by the Plaque Index (PI) (Löe & Silness, 1963) modified, which adopted the same criteria, but evaluating the following 6 teeth surfaces: 16 [buccal (B)], 12 B, 26 [lingual (L)], 36 B, 32 L, and 46 L. According to data distribution, code 0 was considered as no plaque accumulation (PI=0), code 1 for regular plaque accumulation (PI>0 and <1) and code 2 for high plaque accumulation (PI≥1). Individuals were considered positive for gingivitis when all teeth from at least one sextant were affected, with bleeding and inflammation.

The presence or not of any degree of fluorosis was also observed by Dean's index (WHO, 1997).

The stimulated salivary flow rate (SSFR) was measured as previously described (de Almeida et al., 2008), in which volunteers chewed for 5 minutes a 1 cm piece of sterilized latex with a dental floss tied to it to prevent swallowing or aspiration. The flow rates were expressed in mL/min. To classify the SSFR the following numerical scores were attributed: 0 for low caries risk >0.5 mL/min; and 1 for high caries risk ≤0.5 mL/min, because the cut line for dichotomization was based on the data distribution. For the buffering capacity (BC) determination, 3 mL of tritated 5 mM HCl (Merck, Darmstadt, Germany) were added to 1 mL of saliva. The tubes were closed and inverted 5 times, opened and left to stand for 10 minutes (Ericsson, 1959). Final pH of the mixture was determined using a pH-meter and BC

was considered good (score 0) if the final pH was >3.9 and deficient (score 1) for pH≤3.9.

School level (level 2)

Twelve schools were randomly chosen (Kirkwood et al., 1997), being one public and 1 private from each 1 of the 6 health districts studied. It is worth mentioning that, in Brazil, lower income populations have their children attending public schools. Besides the type of school (public or private), it was also verified the presence or not of oral health education programme for the 12 year-old students, permission or not for candy or gum consumption in the classroom and other areas at the institution.

District level (level 3)

It was verified the means of fluoride concentration in water supply of the 6 Health Districts in 2006, which were obtained from data of Municipal Health Secretary of Curitiba and were dichotomized into <0.7 and ≥0.7 mL/L of fluoride in water supply, because it is the minimum concentration allowed in the city.

The classification of the socioeconomic conditions in health districts was based on methodology described by Moraes & Ribeiro (2008), whose authors obtained census data from the Brazilian Institute of Geography and Statistics (IBGE) for the year 2000. It was constructed a socioeconomic score for the health districts with the use of 3 variables: a) percentage of household head with income above 20 minimum wages, b) mean income of the household head, c) percentage of household heads with 17 or more years of schooling. For each one of the three variables, it was calculated the standardized score (*z* score), considering the mean and standard deviation of variable weighted by the size of the district. Afterward, it has been calculated the mean scores for each district. The score was then organized in descending order and grouped into 5 socioeconomic categories. The first category was A (best living conditions) and the last category was E (worst living conditions). Because of data distribution in this study, it was chosen to join B/C as the same group (code 0=better living conditions), and D/E (code 1=worse living conditions) as another group. None of evaluated districts has obtained score A.

Statistical analysis

Firstly, an exploratory study was performed using Fisher and chi-square test. Thereafter, the multilevel software MLwiN version 2.01 (Centre for Multilevel Modelling, Bristol, UK) (Rasbash et al., 2003) was used to estimate the effects of individual, school and district variables on DMFT. The multilevel analyzes were used to determine the relative size of the variance at each level (Leyland & Goldstein, 2001; Hox, 2002). As the outcome was binary, a multilevel logistic model was used. Both the Marginal Quasi Likelihood (MQL) first-order approximation procedures and Predictive Quasi Likelihood (PQL) second-order approximation procedures were used. However, because they produced similar results, only the results based on PQL second-order procedures are reported and discussed (Pattussi et al., 2006). Five models were specified for the outcome. First, a null model, (Model 1), which takes into account the structure from levels 1, 2 and 3. The null model was followed by Model 2 in which variables from level 1 were included. In Model 3, it was included variables from levels 1, 2 and 3. There were adjustments in Models 4 considering: i) all the school and district variables; ii) all the variables at the individual level, which presented *SSA* with the outcome, and iii) interactions at level 1, which have made sense as hypotheses and also demonstrated *SSA*. Interactions at second and third levels did not reach statistical significance and thus were not included in this model. In Model 5, it was included i) all variables at level 1 which showed significance in Model 4 (or any variable that alone did not associate with the outcome, but showed *SSA* within the interactions); ii) all variables at level 2 and 3, and iii) all individual interactions that have made sense as hypotheses and showed significance in Model 4 (fig 2). The results were presented as odds ratios (OR) and their 95% confidence intervals (CI). For each logistic model the intra-class correlation (ICC) was calculated using an approach described by Hox (2002).

Results

Individuals from level 1 (n=687), schools from level 2 (n=12) and districts from level 3 (n=6) were evaluated in this study. Individual and contextual variables and caries status of the students are shown in table 1.

Out of the students, 338 subjects (49.2%) were caries-free (DMFT=0) and 349 (50.8%) had caries experience (DMFT≥1). The mean DMFT for students with caries

experience was 2.88±1.79 and the general mean DMFT was 1.46±1.92, and 1.82±2.05 for public and 1.08±1.70 for private schools.

Of the 687 adolescents who took part in the study, 88.2% were white and 54.9% were female.

In relation to oral health behavior, 93.7% of students reported brushing their teeth twice or more daily, 64.5% used dental floss, and 59.0% used other sources of topical fluoride besides dentifrice at least once a year. In addition, 45.9% looked for a dentist at least once a year and 62.2% used mainly private dental services. In diet, 90.0% of individuals consumed sugar between meals. The individual socioeconomic status was mainly represented by subjects in high-middle class (B1/B2) (45.1%).

In the examination, it was observed that 69.2% of the students demonstrated a regular plaque accumulation (PI>0 and <1) and 58.8% did not have gingivitis. Moreover, 72.3% of the subjects did not show clinical signs of fluorosis.

With respect to salivary parameters, 76.0% showed SSFR above 0.5 mL/min and 97.5% had a good BC.

Firstly, an exploratory study was performed and statistical differences were found (association with caries experience) for the variables ethnic group (light- and dark-skinned black), no flossing, no fluoride use (solution, varnish, gel), public dental access, lower individual socieconomic status, higher plaque index, presence of gingivitis (level 1), and ii) public school, permission for candy or gum consumption (level 2). The variables which not showed statistical association with caries experience were: i) sex, toothbrushing frequency, dental visit frequency, sugar consumption between meals, fluorosis, stimulated salivary flow rate, and salivary buffering capacity (level 1); ii) oral health education (level 2), and iii) fluoride concentration in water supply, and socioeconomic position (level 3).

Table 2 presents the findings of a multilevel logistic regression with outcome variable DMFT=0/DMFT>0. Model 1 (Null Model) showed that there was no statistically significant association (*SSA*) between districts and caries experience (p=0.956). Similarly, no *SSA* was found between schools and the outcome (p=0.250). The variation between districts (7.8%) was much smaller than the variation at the school level (92.3%). The intraclass correlation coefficient for schools was 0.073 and for districts was 0.006. These findings suggest that school and district levels did not differ in caries experience.

In Model 2, it was observed that socioeconomic status (class B, *p*=0.002 and class C, *p*=0.013), public dental access (*p*=0.003), and high PI (*p*=0.014) were associated with caries experience. There was no *SSA* between the lowest socioeconomic status (class D and E) and dental caries experience, probably because of the small number of individuals.

In Model 3, it was verified that the characteristics of schools and districts have not affected caries experience. Significant findings remained similar to those from Model 2.

In Model 4, the lack of association between community-level variables (schools and districts) and caries experience was still maintained. It was found that male sex associated negatively with caries experience (β=-0.709, *p*=0.002). However, the interaction between male sex and no fluoride use was positively associated with caries experience [β=+1.000; *p*=0.002; OR=2.92; 95% CI:1.47-5.80]. Socieconomic class C (*p*=0.045), dental plaque accumulation (regular PI, *p*=0.016 and high PI, *p*=0.000), and fluorosis (*p*=0.026) were significantly associated with caries experience. The interaction between fluorosis and dental biofilm accumulation was negatively associated with caries experience [regular PI (β=-1.265, *p*=0.020) and high PI (β=-2.032, *p*=0.003)]. In addition, the interactions between no flossing and use of public dental services were associated with caries experience [β=+0.809; *p*=0.007; OR=3.79; 95% CI:1.45-9.92] (table 2, fig 2).

All these findings remained significant even after statistical adjustment in the final model (Model 5) (fig 2).

Discussion

Multilevel Modelling is appropriate for analyzing such hierarchical data, and provides an efficient way to link the conventionally distinct populational and individual-level approaches (Leyland & Goldstein, 2001; Hox, 2002). Moreover, it could avoid a loss of statistical power in the use of variables from different levels, and the risk of ecological fallacy, which is a kind of error when only the populational level is considered (Newton & Bower, 2005).

The mean DMFT in the study population was 1.46, considered low by WHO standards and similar to the levels observed in Europe (Petersen, 2003), where the DMFT indexes among 12-yr-old children range from 1.2 to 2.6. However, the mean

DMFT for students with caries experience was 2.88, which demonstrates higher needs for treatment in this population, evidencing the dental caries polarization in the present study. Also, the higher mean DMFT found in public schools reflects the lower socioeconomic status among those students.

Regarding variables from level 1 (individual level), studies suggest that ethnicity seems to be less relevant than socioeconomic status to determine caries outcome (Plamping et al., 1985; Watt & Sheiham, 1999). In Brazil, ethnic differences in dental health may be further due to discrepant socioeconomic status and access to services, with no biological fundament (Antunes et al., 2003).

In this study, male sex was negatively associated with caries experience, differently from the findings by Aida et al. (2008), who did not observe any association between gender and dental caries. Nevertheless, our results corroborate the study realized by Antunes et al. (2006), who argued that an early chronology of permanent tooth eruption in women might determine the higher risk to tooth decay for girls in Brazilians. Moreover, it was reported that girls from “never poor groups” had a higher mean DMFT, which could be explained by higher use of dental care services. Higher access to dental care may lead to an increase in DMFT index, especially regarding the number of filled teeth, as a result of overtreatment (Peres et al., 2007).

The interaction between male sex and no use of fluoride (except for the dentifrice) was positively associated with caries experience, maybe because boys, in general, have a lower care index, which reflects their decreased concern about health and aesthetics (Peres, et al., 2007). In relation to caries-inhibiting effect of fluoride sources, Cochrane systematic reviews found that fluoride rinses are responsible for 26%, fluoride gel for 28%, and fluoride varnish for 46% out of the reduction in the DMFS index (Marinho, 2008), demonstrating the relevance of these topical methods.

Lower individual socieconomic status was statistically associated with caries experience. The relationship between the socioeconomic position of individuals and oral health has been well established. Social class has been significantly associated with high DMFT (Pattussi et al., 2006). Those at the top of the socioeconomic ladder performed better in most health status measurements (Lynch & Kaplan, 2000). The present study also showed that higher degrees of plaque index (regular and high PI) were positively associated with DMFT>0. The relationship between dental biofilm accumulation and dental caries is well known. Caries lesions develop where oral

biofilms are allowed to mature and remain on teeth for long periods (Fejerskov, 2004; Selwitz et al., 2007). In addition, fluorosis was associated with caries experience in this study. Severe fluorosis was seen to increase susceptibility to dental caries and subsurface porosities by hypomineralization (Almerich-Silla et al. 2008). Grobleri et al. (2001) found a strong, positive correlation between caries experience and fluorosis degrees in South African children. Wondwossen et al. (2004), in Ethiopia, also found a positive relationship between caries and fluorosis. Pontigo-Loyola et al. (2007) found more severe caries in those who presented some degree of fluorosis. However, there has been some controversy on whether dental fluorosis increases, decreases, or has no effect on caries risk (Cunha-Cruz & Nadanovsky, 2005). A number of studies have demonstrated that children with fluorosis have lower caries prevalence (Bottenberg et al., 2004; Whelton et al. 2004; Narbutaitè et al., 2007). When plaque index and fluorosis were analyzed in an interaction term, fluorosis seemed to protect against caries only in the presence of dental biofilm accumulation. The interaction observed in this *in vivo* study between fluorosis and dental biofilm accumulation with caries experience has not been carried out in other studies so far. One hypothesis is that fluorotic teeth could release some fluoride ions to the dental biofilm in a low pH condition which, consequently, reduces demineralization. It is known that the availability reduction of some ions in the biofilm, such as fluoride, may increase the cariogenic potential (Margolis and Moreno, 1992; Cury et al., 2000). On the other hand, enamel changes in experimental teeth with signs of mild dental fluorosis that had been exposed to local plaque accumulation were examined *in vitro* and specimens showed signs of surface demineralization under light and scanning electron microscope after a week of exposure (Thylstrup et al., 1990).

Other significant interaction in the present study was shown between no flossing and use of public dental services, which was significantly associated with caries experience. It means an increase in odds of caries experience if these two socioeconomic-dependent variables act together. This finding points to a role of behavior and services use in dental caries outcome, which have also been reported for most chronic diseases (Peres et al., 2007).

Frequency of toothbrushing was not associated with caries experience in this study, contradicting Nguyen et al. (2008), who verified that brushing teeth at least twice a day decreased caries among young adults in Finland. Nevertheless, there is a consensus in the literature that toothbrushing once per day is sufficient to maintain

oral health and to prevent caries and periodontal diseases (Attin & Hornecker, 2005). Also, students' sugar intake between meals was not positively related with their DMFT status. This has been reported previously and might be attributed to the overall high level of sugar consumption (Zero et al., 2004; Kiwanuka et al., 2006).

Interestingly, there was observed no association of salivary flow rate and buffer capacity with caries experience. According to Tenovuo (1997), flow rate has no linear association with dental caries but there seems to exist an individual "threshold" limit, which is decisive for enhancing caries. Besides, salivary buffer capacity has been considered to have only a weak negative association with dental decay (Tenovuo, 1997).

In relation to level 2 (school level), it was not found a *SSA* between the type of school and caries experience. In another Brazilian study, the type of school was not considered a risk indicator of the disease (Cortellazzi et al., 2008). However, it was reported that the public school enrollment was one of the factors identified as determinant of the odds of having one or more untreated decayed permanent teeth (Antunes et al., 2006).

There was no *SSA* between oral health education in schools and dental caries, maybe because of the lack of education programmes considering specific individual needs. In Sweden, an efficient oral health education in schools based on individual self-care reduced the caries incidence (Axelsson, 2006).

It is important to evaluate caries experience not only in large territorial areas, such as states, countries and continents, but also at local and regional levels, for the promotion adequate oral health based on the community needs (Cypriano et al., 2008). With regard to level 3 (district level), it was not found an association between fluoride concentration in water supply and caries experience. Curitiba has a water fluoridation scheme which covers all population since 1958 and a monitoring water fluoridation system since 1995. This homogeneity in the fluoride distribution could be the reason for the lack of association with caries experience. Besides, the 6 evaluated districts present an homogeneous income distribution, which might contribute for the lack of influence in caries experience. However, the reduced number of units (districts) may also be responsible for the lack of power to detect the populational influence.

To our knowledge, the present study is the first one to integrate three hierarchical levels (students, schools, and districts) using a multilevel multivariate

logistic regression model to evaluate biological, behavioral, and contextual determinants in oral health.

The results from a few multilevel studies on dental caries, taking into account two levels, have been reported. Pattussi et al. (2006) surveyed 14/15 year-old students to investigate the association between neighborhood empowerment and dental caries in Brazil, and observed that adolescents from areas with higher levels of empowerment scores had lower levels of dental caries experience. In addition, the study of Tellez et al. (2006) related that features of the neighborhood environments influence the oral health of those individuals exposed to them either in addition or in interaction with individual characteristics among low-income African-American people. Moreover, contextual (human development index and access to fluoridated tap water) and individual sociodemographic (gender, ethnic group, localization and type of school) characteristics influenced dental caries experience in 12-year-old schoolchildren from Brazil when using a conventional multivariate logistic regression (Antunes et al., 2006). In 2007, Turrell et al. analyzed the association of neighborhood disadvantage and individual-level socioeconomic position with self-reported oral health among males and females aged 43–57 years and verified that residents of socioeconomically disadvantaged neighborhoods were significantly more likely to indicate negative impacts of oral conditions on quality of life. In addition, respondents with low levels of education and having a low income household reported poorer oral health independent of neighborhood, demonstrating also individual effects in oral health. Aida et al. (2008) used multilevel analysis to determine the effects of the community context and individual behavior on dental caries and showed that 90.8% of variance in dmft index among Japanese 3-year-old children occurred at the individual level and 9.2% of the variance occurred at the community level.

The main findings were that individual variables had a highly significant association with caries experience even in the presence of the school and district levels (contextual variables).

The risk for caries development varies significantly for different groups. The monitoring of contrasts in dental health outcomes is relevant for programming socially appropriate interventions. The analysis of different risk factors, which influence dental caries, is relevant for planning suitable interventions for target population groups whom could present higher levels of disease. The early

identification of high risk individuals (from polarization groups), could have a pivotal epidemiological impact providing a suitable care for oral health, re-orienting attention on dental prevention. Moreover, an extended knowledge of risk factors for dental caries in different individuals could reduce costs in dental treatments offered by the health systems. Thus, from a cost-effectiveness point of view, caries preventive measures must be integrated and based on predicted risk groups. Overall effective health interventions might be more effective in that part of a population which concentrates disease (Antunes et al, 2006). On the other hand, groups of lower caries risk should not be underestimated for not increasing the probability of presenting the disease, by the fact that risk factors may not be completely recognized and high-risk individuals might not be easy to identify (Batchelor & Sheiham, 2006; Peres et al., 2009). Maybe, a good tool to preserve equity and universality principles for dental caries should be the combination of both polarization and global attention approaches. However, oral health general interventions focused on high-risk and non-vulnerable groups remain a challenge.

The Multilevel Analysis allows the evaluation of different risk factors from distinct hierarchical levels, which makes this approach valuable for the integration of variables influencing the disease outcome, especially for complex diseases, such as dental caries. It was observed that individual factors, such as female sex, the interaction of male sex with no fluoride use, lower socioeconomic status, dental plaque accumulation, fluorosis, and the integration of no flossing with public health service use, further than contextual variables (schools and disctricts), were associated with caries experience in the study population.

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Table 1 Individual and contextual characteristics of students (n=687) and caries status.

Variables	n	Frequency (%)	Proportion of DMFT≥1 (%)
	687	100.0	
Level 1: Student			
Sociodemografic			
Ethnic group			
White	606	88.2	48.5[a]
Light- and dark-skinned black	62	9.0	69.3[b]
Yellow	19	2.8	63.2[a,b]
Sex			
Female	377	54.9	52.2[a]
Male	310	45.1	49.0[a]
Behavioural			
Toothbrushing frequency			
2 or more/day	644	93.7	49.8[a]
Until once/day	43	6.3	65.1[a]
Flossing			
Yes	443	64.5	47.2[a]
No	244	35.5	57.4[b]
Fluoride use (solution,varnish,gel)			
Yes	405	59.0	47.4[a]
No	282	41.0	55.7[b]
Dental visits frequency			
2 times or more/year	304	44.2	48.7[a]
Once/year	315	45.9	50.8[a]
No	68	9.9	60.3[a]
Sugar consumption between meals			
No	69	10.0	50.7[a]
Yes	618	90.0	50.8[a]
Socioeconomic			
Dental access			
Private	427	62.2	41.4[a]
Public	260	37.8	66.1[b]
Individual socieconomic status			
A1/A2 (highest)	175	25.5	32.6[a]
B1/B2	310	45.1	51.9[b]
C	166	24.2	65.1[c]
D/E (lowest)	36	5.2	63.9[b,c]
Clinical			
Plaque Index (modified)			
0	95	13.8	38.9[a]
>0 and <1	475	69.2	50.1[a]
≥1	117	17.0	63.2[b]
Gengivitis			
No	404	58.8	47.0[a]
Yes	283	41.2	56.2[b]

Fluorosis			
No	497	72.3	52.3[a]
Yes	190	27.7	46.8[a]
Stimulated salivary flow rate			
>0.5 mL/min	522	76.0	51.0[a]
≤0.5 mL/min	165	24.0	50.3[a]
Salivary buffering capacity			
pH>3.9	670	97.5	50.9[a]
pH≤3.9	17	2.5	47.1[a]
Level 2: School			
Type of school			
Private	334	48.6	41.3[a]
Public	353	51.4	59.8[b]
Oral health education			
Yes	240	34.9	52.5[a]
No	447	65.1	49.9[a]
Permission for sweet consumption			
Non permitted	228	33.2	37.3[a]
Permitted	459	66.8	57.5[b]
Level 3: District			
Fluoride concentration in water supply			
≥0.7 mL/L	333	48.5	49.2[a]
<0.7 mL/L	354	51.5	52.3[a]
Socioeconomic position			
better living conditions	447	65.1	47.2[a]
worse living conditions	240	34.9	57.5[b]

Fisher's exact or chi-square test. Distinct lower-case superscript letters indicate statistical significance (p<0.05).

Table 2 Multilevel Modelling of 12-year-old students, considering individual, school and district hierarchical levels in relation to caries experience.

	Model 1 (null model)			Model 2			Model 3			Model 4			Model 5		
Variables	***p* value**	**OR**	**CI 95%**	***p* value**	**OR**	**CI 95%**	***p* value**	**OR**	**CI 95%**	***p* value**	**OR**	**CI 95%**	***p* value**	**OR**	**CI 95%**
LEVEL 1: INDIVIDUAL															
Sociodemografic															
Ethnic group															
White (ref)															
Light- and dark-skinned black				0.096	1.66	(0.91-3.02)	0.080	1.71	(0.94 – 3.11)	0.060	1.82	(0.98-3.38)			
Yellow				0.129	2.21	(0.79-6.17)	0.109	2.32	(0.83 – 6.49)	0.137	2.24	(0.77-6.52)			
Sex															
Female (ref)															
Male				0.133	0.77	(0.54 - 1.08)	0.107	0.75	(0.53 – 1.06)	0.002*	0.49	(0.31-0.77)	0.003*	0.51	(0.33 – 0.79)
Behavioural															
Toothbrushing frequency															
2 or more/day (ref)															
Until once/day				0.859	1.07	(0.52-2.21)	0.929	1.03	(0.50 – 2.14)	0.685	0.85	(0.40-1.83)			
Flossing															
Yes (ref)															
No				0.161	1.30	(0.90-1.87)	0.215	1.26	(0.87 – 1.82)	0.189	0.59	(0.26-1.30)	0.635	0.89	(0.56 – 1.42)
Fluoride use (solution, varnish, gel)															
Yes (ref)															
No				0.652	1.09	(0.76-1.55)	0.698	1.07	(0.75 – 1.54)	0.087	0.66	(0.40-1.06)	0.082	0.66	(0.41 – 1.05)
Dental visits frequency															
2 times or more/year (ref)															
Once/year				0.441	0.87	(0.61-1.24)	0.510	0.87	(0.63 – 1.26)	0.922	0.98	(0.68-1.41)			
No				0.614	0.85	(0.46-1.59)	0.736	0.90	(0.48 – 1.68)	0.285	0.70	(0.36-1.35)			
Sugar consumption between meals															
No (ref)															
Yes				0.481	1.22	(0.70-2.11)	0.474	1.22	(0.70 – 2.13)	0.425	1.27	(0.71-2.26)			

Socioeconomic												
Dental access												
Private (ref)												
Public	0.003*	1.91	(1.25-2.92)	0.014*	1.75	(1.12 – 2.75)	0.401	1.26	(0.73-2.19)	0.139	1.47	(0.88 – 2.46)
Individual socieconomic status												
A1/A2 (ref) highest												
B1/B2	0.002*	1.92	(1.27-2.92)	0.009*	1.80	(1.16 – 2.78)	0.099	1.54	(0.92-2.57)	0.009*	1.79	(1.15 – 2.77)
C	0.013*	2.05	(1.17-3.61)	0.038*	1.88	(1.03 – 3.41)	0.045*	2.13	(1.02-4.44)	0.047*	1.83	(1.01 – 3.32)
D/E lowest	0.368	1.50	(0.62-3.60)	0.458	0.40	(0.57 – 3.45)	0.155	2.47	(0.71-8.59)	0.402	1.47	(0.60 – 3.63)
Clinical												
Plaque Index (modified)												
0 (ref)												
>0 and <1	0.151	1.43	(0.88-2.34)	0.130	1.46	(0.89 – 2.38)	0.016*	2.11	(1.15-3.88)	0.008*	2.21	(1.23 – 3.96)
≥1	0.014*	2.28	(1.18-4.40)	0.011*	2.35	(1.22 – 4.56)	0.000*	4.42	(1.97-9.91)	0.000*	4.94	(2.36 – 10.37)
Gengivitis												
No (ref)												
Yes	0.482	1.14	(0.79-1.65)	0.442	1.16	(0.80 – 1.68)	0.397	1.18	(0.80-1.73)			
Fluorosis												
No (ref)												
Yes	0.554	0.90	(0.62-1.29)	0.565	0.90	(0.62 – 1.29)	0.026*	3.03	(1.14-8.01)	0.019*	3.12	(1.21 – 8.08)
Stimulated salivary flow rate												
>0.5 mL/min (ref)												
≤0.5 mL/min	0.732	0.94	(0.64-1.37)	0.729	0.93	(0.64 – 1.37)	0.753	0.94	(0.63-1.40)			
Salivary buffering capacity												
pH>3.9 (ref)												
pH≤3.9	0.484	0.69	(0.25-1.94)	0.543	0.72	(0.25 – 2.05)	0.459	0.65	(0.21-2.03)			
LEVEL 2: SCHOOL												
Type of school												
Private (ref)												
Public				0.894	0.96	(0.54 – 1.70)	0.796	0.92	(0.50-1.69)	0.948	1.02	(0.56 – 1.85)
Oral health education												
Yes (ref)												
No				0.517	0.85	(0.53 – 1.38)	0.850	0.95	(0.56-1.61)	0.676	0.90	(0.54 – 1.50)
Permission for candy or gum consumption												
Non permitted (ref)												
Permitted				0.136	1.51	(0.88 – 2.61)	0.193	1.48	(0.82-2.68)	0.228	1.43	(0.80 – 2.56)

LEVEL 3: DISTRICT										
Fluoride concentration in water supply										
≥0.7 mL/L (ref)										
<0.7 mL/L				0.789	1.06	(0.69 – 1.64)	0.609	1.14	(0.69-1.89)	0.624	1.13	(0.69 – 1.88)
Socioeconomic position										
Better living conditions (ref)										
Worse living conditions				0.623	1.14	(0.68 – 1.89)	0.575	1.16	(0.69-1.97)	0.562	1.17	(0.69 – 1.98)
Interactions										
Male sex and no fluoride use							0.002*			0.003*		
PI score 1 and fluorosis							0.020*			0.018*		
PI score 2 and fluorosis							0.003*			0.002*		
Class B and no flossing use							0.112					
Class C and no flossing use							0.826					
Classes D/E and no flossing							0.269					
No flossing and public access							0.007*			0.028*		
$\sigma^2_{District}$	0.020	0.075	0.020				0.043			0.045		
σ^2_{School}	0.238	0	0				0			0		
$\rho_{District}$	0.006	0.022	0.006				0.013			0.013		
ρ_{School}	0.073	0.022	0.006				0.013			0.013		

** Association with dental caries outcome.*

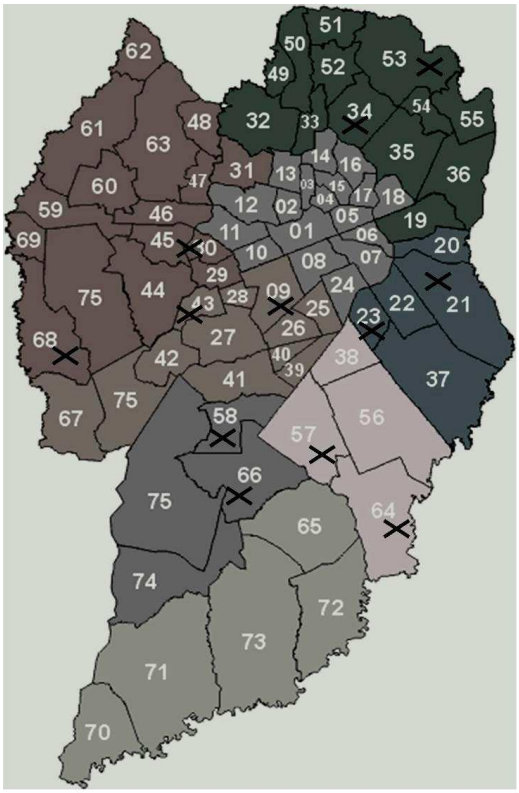

Figure 1 Curitiba map with health districts (grayscale) and neighborhoods (numbers). Twelve schools were randomly selected (marked with X), one public and one private school, from each health district evaluated. Study design was based in municipal data from 2005.

$$\text{cpod}_{ijk} \sim \text{Binomial}(\text{denom}_{ijk}, \pi_{ijk})$$

$$\begin{aligned}\text{logit}(\pi_{ijk}) = {} & \beta_{0jk}\text{cons} + \text{-0,680(0,225)sexo\_1}_{ijk} + \text{0,580(0,223)cceb\_1}_{ijk} + \text{0,603(0,304)cceb\_2}_{ijk} + \\ & \text{0,386(0,461)cceb\_3}_{ijk} + \text{0,791(0,298)ip\_c\_1}_{ijk} + \text{1,598(0,378)ip\_c\_2}_{ijk} + \\ & \text{1,139(0,485)fluorose\_1}_{ijk} + \text{-0,112(0,236)usofd\_1}_{ijk} + \text{0,388(0,262)partsus\_1}_{ijk} + \\ & \text{-0,416(0,239)usofluor\_1}_{ijk} + \text{0,156(0,269)d\_renda\_dic23\_45\_1}_{k} + \\ & \text{0,126(0,257)d\_fluor\_c\_1}_{k} + \text{0,020(0,304)publpart\_1}_{jk} + \text{-0,110(0,263)esb\_1}_{jk} + \\ & \text{0,358(0,297)balach\_1}_{jk} + \text{1,000(0,342)sexo\_1.usofluor\_1}_{ijk} + \\ & \text{-1,260(0,531)ip\_c\_1.fluorose\_1}_{ijk} + \text{-2,012(0,665)ip\_c\_2.fluorose\_1}_{ijk} + \\ & \text{0,809(0,368)usofd\_1.partsus\_1}_{ijk}\end{aligned}$$

$$\beta_{0jk} = \text{-1,459(0,422)} + v_{0k} + u_{0jk}$$

$$\begin{bmatrix} v_{0k} \end{bmatrix} \sim \text{N}(0,\ \Omega_v)\ :\ \Omega_v = \begin{bmatrix} \text{0,045(0,050)} \end{bmatrix}$$

$$\begin{bmatrix} u_{0jk} \end{bmatrix} \sim \text{N}(0,\ \Omega_u)\ :\ \Omega_u = \begin{bmatrix} \text{0,000(0,000)} \end{bmatrix}$$

$$\text{var}(\text{cpod}_{ijk}|\pi_{ijk}) = \pi_{ijk}(1 - \pi_{ijk})/\text{denom}_{ijk}$$

Figure 2 Model 5 observed in MLwin software

Conclusões

4 CONCLUSÕES

Os fatores individuais estiveram associados com a experiência de cárie mesmo com a inclusão de variáveis contextuais na população estudada.

i. o sexo masculino associou-se negativamente à experiência de cárie;

ii. a interação entre o sexo masculino e a não utilização de fluoreto tópico (além do dentifrício) foi positivamente associada com o desfecho de cárie;

iii. posições socioeconômicas mais baixas, acúmulo de placa dental e a fluorose foram associados com a cárie dentária;

iv. a interação entre o acúmulo de biofilme dental e a fluorose foi associada negativamente com a experiência de cárie;

v. a interação entre o não uso de fio dental e a utilização de serviços odontológicos públicos foi associada com a doença cárie.

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Anexos

SALIVARY PARAMETERS, ORAL HEALTH HABITS, AND SOCIOECONOMIC ASPECTS AS RISK FACTORS FOR DENTAL CARIES IN 12-YEAR-OLD CHILDREN FROM A PRIVATE SCHOOL OF THE CITY OF CURITIBA, BRAZIL

Parâmetros salivares, hábitos de saúde bucal e aspectos socioeconômicos como fatores de riscos para cárie dental em crianças de 12 anos de idade em uma escola particular na cidade de Curitiba, Brasil.

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Abstract

OBJECTIVES: The aim of this study was to compare socioeconomic aspects, oral health habits and salivary parameters between 12-year-old students with and without caries experience. MATERIAL AND METHODS: A sample of 113 non-related 12-year-old subjects was selected in a private school of Curitiba-PR, Brazil, for a case-control study. They were divided into groups with and without caries experience through the DMFT index. The frequency of tooth brushing, use of dental floss, diet, frequency of dentist visit, fluoride use, dental plaque index, socioeconomic aspects and salivary parameters were evaluated. Data were analyzed by qui-square and Mann-Whitney. RESULTS: Results showed no statistical difference between the groups in relation to oral hygiene habits and socioeconomic status. It was observed an association between dental plaque presence and caries experience, although not significant ($p = 0.08$). A reduced salivary flow was highly associated with decay experience ($p = 0.00$). CONCLUSION: It was concluded that the salivary flow was an important factor to determine dental caries experience in a homogeneous group of children from a private school of Curitiba-PR. Studies on host response aspects may be desirable in this kind of populations.

Keywords: Dental caries; Risk factors; Salivary flow.

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Analysis of the association between lactotransferrin (LTF) gene polymorphism and dental caries

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Abstract

The present study aimed to evaluate the association between lactotransferrin (LTF) gene polymorphism (exon 2, A/G, Lys/Arg) and dental caries. A convenient sample of 110 individuals, 12 years old, was divided into: *group 1*, 48 individuals without caries experience (DMFT = 0), and *group 2*, 62 subjects with caries experience (DMFT≥1). DNA was obtained from a mouthwash with 3 % glucose solution, followed by a scrapping of the oral mucosa. After DNA purification, polymerase chain reaction (PCR), single strand conformation polymorphism (SSCP) was performed to access the study polymorphism. The LTF A/G (Lys/Arg) polymorphism had been previously reported as located in exon 1. In this study, the referred polymorphism was shown to be present in exon 2. There were no statistically significant differences in the LTF allele (p=0.07) and genotype (p=0.19) frequencies between groups with and without dental caries experience. However, allele 2 frequency was higher in group 2, which suggests an association with disease experience. Lactotransferrin A/G (exon 2, Lys/Arg) polymorphism was weakly associated with susceptibility to dental caries in 12-year-old students.

Key Words: Caries experience, LTF, gene polymorphism, exon 2.

Short Title: LTF gene polymorphism and dental caries.

PUCPR

Análise Multinível de fatores sociais e biológicos associados à cárie dentária

Cód do voluntário:

Data:	Duplicata INTER:	Duplicata INTRA:	Cód examinador:
Nome:			Série e turma:
Cidade de nascimento:		Sexo:	Grupo Étnico (B, N, M, A):
Data de nascimento:			Idade em anos:
Endereço:			Bairro:
Telefone(s) para contato:		Cidade:	
Escola:		Tipo:	D.S.:

Índice de Placa (IP) Modificado

16V	12V	26P
46L	32L	36V

IP=

Características:

0 – **ausência** de placa
1 – **visualização** da placa com **uso de sonda** periodontal
2 – placa **clinicamente visível**
3 – placa **abundante**

CPO-D

17	16	15	14	13	12	11	21	22	23	24	25	26	27
47	46	45	44	43	42	41	31	32	33	34	35	36	37

CPO-D:

Códigos Diagnóstico CPO-D:

0 – Hígido
1- Cárie
2- Restaurado com cárie
3- Restaurado sem cárie
4- Perdido por cárie
5- Perdido por outras razões
6- Selado
7- Suporte para prótese, coroa protética ou faceta / implante
8- Dente não irrompido
9- Dente excluído
10- Mancha branca *ativa*
11- Mancha branca *paralisada*
12- Erosão
T – Trauma (fratura)

FLUOROSE

( ) SIM – grau e região:_____ ( ) NÃO

GENGIVITE

( ) SIM – grau e região:_____ ( ) NÃO

PERIODONTITE

( ) SIM – grau e região: _____ ( ) NÃO

ANÁLISE BIOQUÍMICA

Fluxo salivar:_____
Capacidade tampão:_____
pH salivar:_____

Data:	
Nome do responsável pelo aluno:	
Estado civil:	
Nome do aluno (a):	
Endereço:	
	Bairro: Cidade:
Escola onde o aluno(a) estuda:	

QUESTIONÁRIO A SER RESPONDIDO PELOS RESPONSÁVEIS DOS ALUNOS

Número de pessoas que moram na sua casa (inclusive você): _____

Posse de itens (quantos)

ITEM	***QUANTIDADE***
Televisão em cores	
Rádio (vale também *microsystem* ou rádio tipo *walkman*)	
Banheiro	
Automóvel	
Empregada mensalista (que trabalha pelo menos 5 dias por semana)	
Aspirador de pó	
Máquina de lavar roupa	
Videocassete e/ou DVD	
Geladeira duplex (com duas portas)	
Geladeira simples (não duplex)	
Freezer	

Assinale com um "X" o <u>maior</u> grau de instrução da mãe e do pai do aluno

	MÃE	**PAI**
Analfabeto		
Primário incompleto		
Primário completo		
Ginasial incompleto		
Ginasial completo		
Colegial incompleto		
Colegial completo		
Superior incompleto		
Superior completo		

Primário – 1ª a 4ª série do 1º grau (ensino fundamental)

Ginasial – 5ª a 8ª série do 1º grau (ensino fundamental)

Colegial – 1ª, 2ª e 3ª séries do 2º grau (ensino médio)

Superior – faculdade

Perguntas sobre a saúde bucal do seu filho (a)

1) Seu filho (a) apresenta algum problema de saúde?

sim ( ) não ( ) Qual?_____

2) Seu filho (a) toma algum remédio?

sim ( ) não ( ) Qual?_____

3) Seu filho (a) tomou antibiótico nos <u>últimos três meses</u> ? (exemplos de antibióticos: penicilina, amoxicilina)

sim ( ) não ( )

4) Quantas vezes seu filho (a) escova os dentes por dia?

nenhuma vez ( ) uma vez ( ) duas vezes ( ) três vezes ( ) quatro ou mais vezes ( )

5) Seu filho (a) usa fio dental?

sim ( ) não ( )

5.1) Caso a resposta anterior tenha sido **<u>sim</u>**, relate quando seu filho (a) usa fio dental:

todos os dias ( ) de vez em quando ( )

6) Quantas vezes seu filho (a) ingere açúcar (salgadinhos, bolachas, lanchinhos), por dia, fora das refeições?

nenhuma vez ( ) até 3 vezes ( ) mais de 3 vezes ( )

7) Quantas vezes ao ano seu filho (a) vai ao dentista?

nenhuma vez ( ) 1 vez ( ) 2 vezes ou mais ( )

8) Seu filho (a) freqüenta:

dentista particular ( ) dentista do Posto de Saúde ( )

9) Seu filho (a) usa flúor?

sim ( ) não ( )

9.1) Caso sua resposta anterior tenha sido **<u>sim</u>**, relate quando seu filho (a) utiliza flúor:

todos os dias ( ) de vez em quando ( ) só no dentista ( )

9.2) Qual forma seu filho (a) utiliza o flúor:

bochecho ( ) gel ( ) verniz ( )

10) Seu filho (a) bebe água **<u>mineral</u>** com freqüência?

sim ( ) não ( )