

Cariogenic Microorganisms and Oral Conditions in Asthmatic Children

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Key Words

Asthma • Biofilm • Dental caries • Drug therapy •
Lactobacillus • *Streptococcus mutans*

Abstract

This study evaluated the caries risk of asthmatic patients on the basis of mutans streptococci (MS) and lactobacilli levels in saliva samples as well as the index of oral hygiene and dental caries (DMFT index). The study population was composed of 80 asthmatic children, aged 3–15 years, who use specific medication, and 80 matched, healthy control children. The parents were interviewed about oral health-related factors. The World Health Organization criteria were used for dental examinations. The Köhler and Bratthal methodology was used to detect salivary MS levels and dilutions of saliva were done for lactobacilli counting. No differences between asthma and control groups were observed for caries prevalence in children aged 3–6 and 7–10 years, except in severe cases in the younger group. However, higher caries prevalence for permanent dentition was observed in 11- to 15-year-old asthmatic children. An increased dental biofilm was observed in the asthma group, as well as salivary levels of MS. No differences were observed in levels of lactobacilli.

No statistical correlations were found between medication, frequency of treatment, method of consumption and caries experience, dental biofilm and salivary levels of MS or lactobacilli. However, there was a correlation between MS levels and treatment duration. The logistic regression revealed that MS level is an important risk factor for increased caries experience. Asthma should be evaluated as a risk factor for caries experience because it can increase the levels of MS and the dental biofilm.

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Oral health and prevention of oral diseases are recommended by the World Health Organization [1997]. It is necessary to identify predictive factors of diseases when planning programs related to oral health [Watt, 2005].

This study investigated the influence of bronchial asthma, which is one of the most prevalent childhood diseases, on oral health. In the United States, besides dental caries, asthma is the most common chronic disease [Shulman et al., 2001].

The treatment of asthma can be divided into two phases: the first is to control the symptoms of the acute phase, such as bronchospasm, with the use of bronchodilator

medicines. The second phase is to prevent new acute events through maintenance treatment, by using inhaled or systemic steroids and bronchodilators of long duration [Rang et al., 2003].

Some authors found that asthmatic subjects could have higher caries risk, paying special attention to the medication used [Ryberg et al., 1987], however, other authors did not observe the same results [Meldrum et al., 2001]. Increased caries experience was associated with prolonged use of bronchodilators, which leads to reduced salivary flow [Ryberg et al., 1987]. Reduced salivary flow is accompanied by a concomitant increase in cariogenic microorganisms, lactobacilli and *Streptococcus mutans* in the oral cavity, which are contributing factors for higher caries rates. In addition, antiasthmatic medications containing fermentable carbohydrates and sugars can indirectly decrease oral pH, another factor that can contribute to the development of the disease [Reddy et al., 2003]. Also, the occurrence of dental enamel defects (enamel opacity) correlated with greater asthma severity [Guergolette et al., 2009].

There are many factors related to dental caries etiology, such as diet, salivary flow, salivary components, host defenses, and socioeconomic conditions. Considering the importance of an early identification of populations at risk of dental caries, this study intended to evaluate whether the asthmatic population needs special dental attention in the form of prevention programs.

The present study evaluated the salivary levels of mutans streptococci (MS) and *Lactobacillus* sp., the pattern of oral hygiene and the occurrence of caries disease in asthmatic children. There are few studies evaluating the microorganisms in asthmatic subjects and, because of controversies in the literature about asthma and caries, we also verified the potential associations between pharmacotherapy in asthmatic and nonasthmatic children from ages 3 to 15 years in the city of Londrina, Brazil.

Patients and Methods

A cross-sectional case-control study was performed with children and adolescents aged 3–15 years. All of the participants resided in the city of Londrina, PR, Brazil, which has a concentration of fluoride in the water of 0.77 ppm. The asthma group (n = 80) consisted of children and adolescents selected from patients treated via the Programa Respira Londrina (Breath Londrina Program) of the Londrina State University Hospital. All asthmatic subjects were either under continuous treatment with corticosteroids or were using bronchodilators.

The subjects forming the control group (n = 80), matched for age and gender, were selected from Londrina public schools, and

were not using chronic medication. Also, both groups should not have any other systemic diseases and were not using any other medication that could modify the salivary parameters (such as antibiotics) at least 30 days before the study.

The experimental protocol was approved by the Research Ethics Committee (University of North of Paraná, UNOPAR, number PP/0140/06). The children's parents or legal guardians received information about the purpose of this research and informed consent was obtained prior to the clinical examination and saliva collection. All children received instructions about oral health maintenance and a toothbrush at the end of the clinical examination. Only children whose guardians had signed the informed consent were enrolled in this study.

The parents were interviewed about personal data, medical and dental history of their children, with special regard to bronchial asthma history. These data were used to classify asthma severity, which was confirmed through subjects' medical records. Parents were asked about asthma-related hospitalizations, acute outpatient visits, and episodes of wheezing in the past 12 months; what drugs the children were taking and the duration. The classification for severity of asthma followed the criteria of Shulman et al. [2001]: (a) severe: 2 hospitalizations or 4 asthma-related acute visits; (b) moderate: 1 hospitalization or 2 acute visits or 3 episodes of wheezing; (c) mild: no hospitalizations or 1 asthma-related acute visit or 2 episodes of wheezing; (d) controls: no reported physician-diagnosed asthma.

A pilot study was previously completed using 26 children for validation of the questionnaire and the methodology, as well as the examiner's calibration. The kappa intraexaminer's value was 0.98 and interexaminer's value was 0.96.

Saliva samples for microbiologic analysis were collected at the Dental Clinic of North Paraná University, UNOPAR. Every subject chewed a Parafilm 'M' pellicle (Laboratory Film; American National Can., Chicago, Ill., USA) of 3.0 × 3.0 cm size, for 1 min, without swallowing, to stimulate salivation. For quantification of lactobacilli, 1 ml of saliva was collected with the aid of sterilized disposable syringes. The samples were diluted at the Microbiology Laboratory of UNOPAR, soon after collection, until 10⁻⁴ dilution in brain heart infusion broth (Difco, Becton Dickinson, France SA, France). After that, 50 µl of the dilution was spread onto the selective medium Rogosa agar (Difco). The plates were incubated in anaerobiosis jars (Permutation, Curitiba, PR, Brazil), using the candle method to allow the generation of carbon dioxide inside the jar, at 37°C for 48 h.

Lactobacilli forming colonies were counted by means of an electronic colony counter (Phoenix model CP 602, São Paulo, SP, Brazil), in colony-forming units per milliliter (CFU/ml). The number of colony-forming units was counted and compared with the criteria described by Krasse [1988], in which there was a high risk of decay when there were more than 100,000 CFU/ml and a low risk to decay when there were less than 1,000 CFU/ml.

In order to estimate MS levels, bacterial samples from the children's oral cavity were collected through the wooden spatula sampling technique described by Köhler and Bratthal [1979]. Subjects chewed the film again for 1 min without swallowing, in order to remove the biofilm from the teeth. About 30 mm of the spatula was introduced into the mouth of children and pressed 10 times (5 on each side) on the dorsal surface of the tongue to contaminate it with saliva. When removing the spatula from the mouth, subjects were asked to close their lips, with the purpose of removing excess saliva.

Each side of the spatula was pressed against a different area of a 67 × 15 mm crosshatched Petri dish (Rodac plate type, Inlab-Interlab of Scientific Products Ltda, São Paulo, SP, Brazil), containing mitis-salivarius agar (Difco). This medium was added to 15% of sucrose (Difco), 0.2 units/ml of bacitracin (Bik Química Farmaceutica Ltda, São Paulo, SP, Brazil) and 1 ml of 1% potassium tellurite solution (Chapman solution) for 1 liter of culture medium, according to Gold et al. [1973], to promote a selective medium for MS.

The agar plates were incubated at 37°C for 48 h in anaerobic jars with a candle to generate CO₂. By using the same electronic counter, the number of colony-forming units of MS on a predetermined square of the tip of the spatula (1.5 cm²) was counted for each side pressed against the surface agar. From each impressed site, the square with the larger number of colonies was chosen. The mean colony-forming units of the two sides of the spatula (CFU/spatula) were determined and the counts were interpreted according to the criteria described by Köhler and Bratthall [1979]: 0–20 CFU, corresponding to 0–10⁴ CFU of MS/ml of saliva, representing low caries risk; 21–100 CFU, corresponding to 10⁵–10⁶ CFU of MS/ml of saliva, representing moderate caries risk; >100 CFU, corresponding to >10⁶ CFU of MS/ml of saliva, representing high caries risk.

Oral health status, caries experience, caries severity and the biofilm index were evaluated in children using a calibrated examiner and with another person's help to record the data. The exams were done with the aid of artificial light and plain mirrors. Caries experience and severity were observed according to World Health Organization [1997] criteria, through DMFT (decayed, missed and filled teeth) index. In doubtful cases, an explorer with a round tip was used to facilitate the diagnosis. The biofilm index was measured by using the Simplified Oral Hygiene Index (SOHI) developed by Greene and Vermillion [1964]. The teeth were colored using 2% fuchsin to verify SOHI, and then the children were given oral hygiene and cleaning instructions.

The Statistical Package for Social Sciences 15.0 (SPSS, London, UK) was used to calculate the frequency of the population data and to correlate factors such as DMFT, biofilm index, microorganism levels, medications and methods of consumption, and to compare the two groups. The χ^2 test was used to assess associations in categorical data between groups. All variables were compared between the two groups by means of Kruskal-Wallis and Mann-Whitney tests. The critical level for statistical significance was $p < 0.05$, with a confidence interval of 95%.

Logistic regression was used in order to assess the risk of caries (DMFT index as a dependent variable) in a multivariable analysis. The model included the variables asthma or control, salivary MS levels (and categories), levels of lactobacilli (and categories) and biofilm index (and categories) adjusted for age.

Results

One hundred and seventy-six children were recruited for this research, but 16 of them were excluded because of the following reasons: the amount of saliva collected was not enough to accomplish the microbial dilution procedures, contamination of saliva samples at the laboratory, as well as not showing up for the required dental appoint-

Table 1. Distribution of study population of asthmatic (n = 80) and control groups (n = 80) according to age, gender, dentition, caries experience, salivary levels of MS and lactobacilli, and dental biofilm index (SOHI)

Characteristics		Number	Percent
Child's age	3–6 years	39	24.4
	7–10 years	73	45.6
	11–15 years	48	30.0
	total	160	100.0
Gender	female	91	56.9
	male	69	43.1
	total	160	100.0
Dentition	deciduous	27	16.9
	mixed	99	61.9
	permanent	34	21.3
	total	160	100.0
Caries experience	no	59	36.9
	yes	101	63.1
	total	160	100.0
MS levels	low	63	39.4
	medium	69	43.1
	high	28	17.5
	total	160	100.0
Lactobacilli levels	low	58	36.3
	high	102	63.8
	total	160	100.0
SOHI	satisfactory	64	40.0
	regular	79	49.4
	insufficient	17	10.6
	total	160	100.0

ment. Therefore, 160 subjects were enrolled in this study, 80 asthmatic (test group) and 80 nonasthmatic (control group) children.

The distribution of the characteristics of test and control groups is shown in table 1, with the variables age, gender, dentition, caries experience, salivary MS levels, levels of lactobacilli and dental biofilm index (SOHI). Asthma severity, type of medication used, frequency of use, method of consumption and duration of asthma treatment in the test group are displayed in table 2.

There was no significant difference between the caries experience of the two groups – asthma and control groups – by means of χ^2 test ($p > 0.05$).

Table 2. Distribution of asthmatic population (n = 80) according to asthma severity, medication used, frequency of use, method of consumption and duration of the treatment against asthma

Characteristics		Number	Percent
Group	control	80	50.0
	asthma	80	50.0
	total	160	100.0
Asthma severity	intermittent	13	8.1
	mild	17	10.6
	moderate	29	18.1
	severe	21	13.1
Medication	bronchodilator	52	32.5
	corticoid	13	8.1
	bronchodilator + corticoid	15	9.4
Frequency of use	no	4	2.5
	acute crisis/irregular use	41	25.6
	continuous use	34	21.3
Method of consumption	oral	49	30.6
	inhalation	20	12.5
	oral + inhalation	11	6.9
Treatment duration	<2 years	27	16.9
	2–4 years	22	13.8
	>4 years	31	19.4

Table 3. Distribution of asthmatic children according to salivary MS levels and asthmatic treatment duration

	Equivalent MS levels, CFU/ml ^a			
	≤10 ⁴	10 ⁵ –10 ⁶	>10 ⁶	total
Duration of asthma treatment*				
<2 years	12 (44.4)	11 (40.7)	4 (14.8)	27 (100.0)
2–4 years	10 (45.5)	11 (50.0)	1 (4.5)	22 (100.0)
>4 years	9 (29.0)	8 (25.8)	14 (45.2)	31 (100.0)
Total	31 (38.8)	30 (37.5)	19 (23.8)	80 (100.0)

^a Köhler and Bratthall [1979].

* Statistically significant, by Spearman correlation, $p = 0.026$. Figures in parentheses are percentages.

When these groups were stratified by age (3–6, 7–10 and 11–15 years), a higher caries prevalence was observed in the asthma group (DMFT 2.11 ± 0.36) than the control group (DMFT 1.05 ± 0.31), for permanent teeth, in children 11–15 years old, by means of Mann-Whitney test ($p = 0.024$) (fig. 1). The results in parentheses are means $\pm$ standard error (SE).

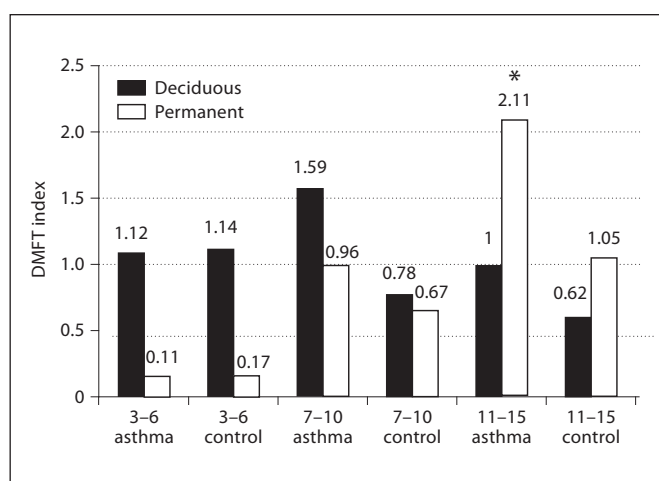


Fig. 1. Caries experience (DMFT index) of children in the three age groups, of deciduous and permanent groups and asthma and control groups. *Statistical difference between asthma and control groups at the age of 11–15.

There was a statistical difference in the biofilm index between the asthma (index mean $\pm$ SE: 1.47 ± 0.06) and control groups (mean $\pm$ SE: 1.27 ± 0.07) using the Mann-Whitney test ($p = 0.047$).

When evaluating the target microorganisms, significantly higher levels of MS were observed in the asthma group (mean $\pm$ SE: 70.40 ± 8.95 CFU/spatula) in relation to the control group (mean $\pm$ SE: 44.20 ± 4.81 CFU/spatula) using the Mann-Whitney test ($p = 0.04$). On the other hand, no significant difference was observed in the lactobacilli counts in the asthma group (mean $\pm$ SE: $124.1 \pm 13.37 \times 10^3$ CFU/ml) in relation to the control group ($119.35 \pm 14.27 \times 10^3$ CFU/ml) according to the Mann-Whitney test ($p = 0.75$).

We observed a significant correlation between MS levels and the biofilm index ($r_s = 0.18$, $p = 0.02$) in the whole population, however, within each study group, we found a correlation between MS levels and the biofilm index only in the asthma group ($r_s = 0.22$, $p = 0.04$).

The correlation between MS and caries experience in the population was positive ($r_s = 0.24$, $p = 0.002$), and there was also a positive correlation between MS counts and caries experience in the asthma group ($r_s = 0.23$, $p = 0.04$) and in the control group ($r_s = 0.24$, $p = 0.02$).

The pharmacotherapy of the asthma group was also evaluated but a significant correlation was only found between MS levels and the duration of the treatment ($r_s = 0.25$; $p = 0.026$) (table 3). Significant correlations were not observed between the type of medication, frequency of

Table 4. Logistic regression of the variables asthmatic group (yes/no), MS levels, lactobacilli levels, biofilm index (SOHI) and age over the dependent variable caries experience

Variable	B ^a	SE	Wald	p	R ²	p(R ²)
Asthmatic group (yes/no)	-0.2388	0.353597	0.456088	0.499		
MS levels	0.007564	0.003486	4.708112	0.030*		
Lactobacilli levels	0.001629	0.001454	1.256313	0.262	0.13	0.008
SOHI index	-0.10904	0.294805	0.136795	0.711		
Age	0.127344	0.059384	4.598593	0.032*		

^a B = Variable coefficient. * Statistical significance of MS levels and age over caries experience.

use and method of consumption of medicaments with the clinical parameters caries experience and oral hygiene, or levels of MS and lactobacilli.

The logistic regression revealed that the MS level and age were important risk factors for caries experience in the whole population (table 4).

Discussion

There is some controversy in the scientific community about the relationship between asthma and caries, and regarding the different methodologies used to study these diseases. In this study, widely known indexes and methodologies were used, that allowed comparison between groups with or without asthma.

The risk of dental caries in people that use asthma medications regularly has been well documented and its occurrence is mainly due to alterations in salivary parameters [Kenny and Somaya, 1989; Maguire et al., 1996; Paganini et al., 2011]. Asthma itself was considered a risk factor for dental caries by some authors [Kankaala et al., 1998; Ersin et al., 2006; Anjomshoaa et al., 2009; Stensson et al., 2010], or the medication used for asthma treatment was blamed [Ryberg et al., 1991; Kankaala et al., 1998; McDerra et al., 1998; Reddy et al., 2003; Wogelius et al., 2004; Ersin et al., 2006; Milano et al., 2006; Shashikiran et al., 2007]. But there are other authors who did not find a correlation between asthma and/or the medication used and an increased caries risk [Meldrum et al., 2001; Shulman et al., 2001].

In the present study, the data showed that there was no difference in caries experience between asthma and control groups, in children aged 3–6 and 7–10, which is in agreement with the observations of Ryberg et al. [1987], Shulman et al. [2001], Meldrum et al. [2001] and Eloit et

al. [2004]. We only found an association between asthma severity and caries experience in 3- to 6-year-old children. Reddy et al. [2003], Ersin et al. [2006] and Shashikiran et al. [2007] also found an increase in caries prevalence with an increase in the severity of asthma.

On the other hand, we observed a higher level of caries experience in the 11- to 15-year age group, in the asthma group compared with controls, but only in permanent teeth. These data are in agreement with the work of Ryberg et al. [1991], McDerra et al. [1998] and Wogelius et al. [2004], who found larger caries prevalence in asthmatic children in permanent molars. However, Wogelius et al. [2004] pointed out the possibility that these teeth could be influenced by enamel hypomineralization. Mehta et al. [2009], similar to the present work, showed higher prevalence of caries among asthmatic subjects aged 11–25, as well as a correlation with the duration of asthma. These observations suggest a cumulative effect of this disease and its influence on oral health.

The control group presented better conditions of oral hygiene, as evaluated through the bacterial biofilm index [Greene and Vermillion, 1964]. However, it was observed that the pattern of oral hygiene was not influenced by the pharmacotherapy of asthma in this study population. Our data are in agreement with the study by McDerra et al. [1998], who found differences in the biofilm index, where children with asthma had a higher biofilm index than the control group. On the other hand, some authors did not observe differences in oral hygiene patterns between the asthma group and the respective controls [Ryberg et al., 1987; Eloit et al., 2004; Ersin et al., 2006].

This correlation of asthmatic children with more dental biofilm is of special interest because there are many different species in a cariogenic biofilm. According to Beighton [2005], the species of *Bifidobacterium* and *Acti-*

nomyces can also be involved in the etiology of dental caries, not only lactobacilli and MS.

Despite the importance of the cariogenic biofilm in asthmatic subjects and the many species that harbor it, we decided to investigate MS and lactobacilli levels because of their role in caries development, and because of the feasibility of methods to isolate and identify these bacteria.

In this study, we observed a higher number of MS in asthmatic children than in the control group, however, there was no difference between the groups for lactobacilli levels. Similar data were observed by Ryberg et al. [1987], who found higher levels of MS in the children of the asthma group than in the controls, but no differences in lactobacilli numbers. The same authors, 4 years later, found larger levels of lactobacilli in the saliva of children with asthma in relation to the control group [Ryberg et al., 1991].

In agreement with Ersin et al. [2006], we also found that the number of MS increased with the duration of asthma treatment. In this study there was a positive correlation between the number of MS and the biofilm index, MS and dmst and DMST, besides MS and the duration of the treatment.

Although Reddy et al. [2003] reported an increased level of lactobacilli and MS in the oral cavity of asthmatic subjects using bronchodilators, we did not observe, in our study, an association with the class of drugs, the beginning of treatment, the frequency of use, method of consumption and caries experience, the pattern of oral hygiene and MS and lactobacilli levels. We observed an increase in the salivary levels of MS, correlated with asthma treatment duration, which can be attributed to a decrease in salivary flow and, therefore, an indirect effect of medication [Shashikiran et al., 2007]. The salivary flow rate of subjects of the present study was investigated by Paganini et al. [2011]. They found that children with moderate or severe asthma showed reduced salivary flow, as seen by Shashikiran et al. [2007].

There are few reports on oral alterations related to dental caries in children and adolescents with asthma, mainly concerning the quantification of the most important cariogenic microorganisms MS and lactobacilli. The differences found in the pattern of oral hygiene (represented by the biofilm index) and in the salivary levels of MS in the asthma group, as well as the correlation between the duration of asthma treatment and higher MS levels can contribute to the design of prevention programs. Dental caries is a multifactorial disease and extra attention should be given to asthmatic subjects regarding this, because an increased risk exists for the development of caries lesions, represented mainly by an increase in MS levels.

In conclusion, asthmatic children and adolescents presented greater caries prevalence in permanent teeth after 10 years of age, as well as poor oral hygiene and greater MS salivary levels, an important bacterial group involved in the etiology of caries. Besides other important factors that should be evaluated, our results suggest that asthmatic children and adolescents should receive special dental attention for the prevention of caries.

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Disclosure Statement

There are no conflicts of interest in this paper.

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