Changes in Bacterial Profiles After Periodontal Treatment Associated with Respiratory Quality of Asthmatic Children.

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Changes in bacterial profiles after periodontal treatment associated with respiratory quality of asthmatic children

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Abstract

Background Despite the reduction phenomenon of asthma exacerbation after dental plaque control, no scientific report has been found to describe the link between bacterial profiles and respiratory quality in children with asthma.

Objective To investigate association between bacterial profiles changes and improvement in respiratory quality after periodontal treatment.

Methods Asthmatic children with FEV1 reversibility ≥12% and dental plaque index ≥2 who qualified for inclusion criteria were randomized into two groups. The treatment group was referred for dental plaque removal by oral biology dentist and guided to perform an individual oral health care for seven days. The control group was observed without intervention. Each subject was assessed for respiratory quality and bacterial profiles taken from plaque culture before and after one week run-in period. Paired t-test and correlation were used for statistical anayses. The study protocol was approved by the Medical Research Ethics Committee of Dr. Soetomo Hospital.

Results Dental plaque control was performed in 18 of 36 children with mild asthma. At follow-up, plaque analysis among the subjects receiving dental treatment showed a significant reduction ($P<0.01$) in number of microbial colony and gram negative bacilli, corresponding by a fall in asthma score, FEV1 reversibility, and blood eosinophil ($P<0.01$). The improvement of respiratory quality variables were moderately associated ($r>0.4$; $P<0.05$) with bacterial profiles changes after periodontal treatment.

Conclusions A reduced rate of gram negative bacilli colonization in dental plaque after periodontal treatment is related to improvement of respiratory quality of asthmatic children. **[Paediatr Indones. 2008;48:327-37]**.

Keywords: *asthma, dental plaque, FEV1 reversibility, bacteria*

Despite much progress in our understanding of asthma mechanisms over the past decade, up to 40% of patients remained symptomatic with the current multidisciplinary treatment approach to asthma management.[1] Educational aspects and attention to avoidance of agents that may trigger asthma attacks are essential whilst conventional drugs in acute care settings remain the cornerstone of treatment.[2] While in recent year, there has been resurgence of interest that oral infections such as periodontal diseases may contribute to the systemic chronic inflammation burden,[3-5] our preliminary study has added significant evidence which suggested that aggressive removal of dental plaque may decrease asthma severity and

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frequency of exacerbations. Mechanical debridement provided by dentist followed by daily regular tooth brushing and gargling of 0.1% hexetidine, had successfully ameliorate the hyperactive airways. After one week of follow up, there were improvement of lung function parameters equivalent with 55% fall in FEV1 reversibility, concomitant with 93% reduction in asthma symptoms. However, it failed in establishing any causal relationships between dental plaque exposures and asthma.[6]

Dental plaque, as a tightly adherent biofilm of the tooth surface, consists of approximately 200-500 species of microorganisms enmeshed in a complex mixture of micro flora, with representative bacterial level from more than 10^{11} cfu/ml.[7] Available scientific evidence suggests that metastatic inflammation caused by oral microorganisms may be a risk for the occurrence of systemic diseases,[8] we hypothesize that bacterial dental plaque may induce immunological injury at the airway and trigger asthma symptoms. Control of dental plaque formation has been shown to effectively eliminate bacterial accumulation, inhibit the progression of periodontal disease, decrease the systemic chronic inflammation burden caused by oral inflammation,[7-8] and may contribute to the reduction of asthma exacerbation.[9] However, there is still too little evidence to draw this conclusion and the concept remains controversial. To find out the association between changes of bacterial profiles and respiratory quality, we performed microbial diagnosis and monitored asthma parameters before and after periodontal treatments.

Methods

Design

This was a randomized, parallel group, clinical trial which took place on Allergy Immunology Outpatient Clinic, Department of Child Health Dr. Soetomo Hospital, Surabaya during July-September 2007. The study protocol was reviewed and approved by the Medical Research Ethics Committee of Dr. Soetomo Hospital. Each parent was given an information sheet to read and asked to sign a written consent. Each child was asked for verbal assent or consent.

Subjects

Children aged 6-12 year-old with asthma criteria as determined by PNAA 2004[10] for ≥ 3 months were recruited if qualified for a prebronchodilator forced expiratory volume in one second (FEV1) ≤ 75% of Polgar predicted normal value or had FEV1/FVC ratio < 80% (if FEV1 > 75%) at Visit 1; met the reversibility criteria on the basis of an increase in FEV1 ≥ 12% from prebronchodilator FEV1, 15-30 minutes after two actuations of a Salbutamol pMDI, performed at Visit 1; with dental plaque index (DPI) ≥2 using Silness and Loe scoring method;[11] and had willingness to participate as indicated by signing informed consent form. We excluded subjects who had any teeth decays, severe periodontitis, severe structural abnormalities of mouth and teeth, use of orthodontic or prosthesis instrument; any acute exacerbation of asthma or clinically relevant mouth, throat, and upper respiratory infections within one month prior to Visit 1; past or present hemostasis disorder, diabetes mellitus, congenital heart disease, rheumatic heart disease, hypertension, hepatitis, cirrhosis, tuberculosis, malignancy, HIV infection, convulsive disorder, mental retardation, attention deficit hyperactivity disorder (ADHD), autism, obesity, and any medical conditions considered that may interfere or put subject at risk because of participating in the study; under care by respirologist or allergy specialist for immunotherapy; use of antibiotics, oral or parenteral steroids, long acting β-agonist, antileukotriene modifiers, anticholinergics, xantine, cromoline, or antihistamine during at least the preceding two weeks prior to Visit 1 or any time during the study; any history of smoking.

Subjects were subjected for discontinuation from this study at any time for specific reasons: decrease in FEV1 ≥ 25% from Visit 1 or < 40% of predicted; had an acute exacerbation of asthma that need the use of asthma controller medication; taken any form of medication that could affect their periodontal status, such as antiinflammatory agents, antibiotics and immunosuppressant during the study period, or noncompliance with the oral hygiene procedure.

Study protocol

Eligible subjects were randomized to either receive treatment or nothing. Randomization was done

using systematic random sampling. Sample size was calculated based on the formula for difference between proportions for independent groups with a power of 80%. After written informed consent was obtained, each subject in both groups was initially assessed and scheduled for evaluation after one week run-in period. Dental plaque index was visually examined, and two set of dental plaque swabs were collected from all subjects before and after study procedure. The specimens then were cultured for bacterial colonies counting and identification after 24 hours of collection. Respiratory quality variables consisted of asthma score, lung function recorded by spirometry, and blood eosinophil were measured at each visit.

Periodontal treatment

The periodontal procedure for the treatment group was first initiated by oral biology dentist at the first visit, consisted of dental plaque removal by mechanical debridement using rotating brush, pumice, combine with sickle-shaped explorer for 'assisted drainage method'.[12] Subjects in the treatment group were guided to perform individual dental plaque control for seven days at home by regular tooth brushing and gargling of 0.1% hexetidine at least twice daily. Simple education about oral health behavior was recommended, including diet manipulation to eliminate any foods and/or drinks containing fermentable or nonfermentable sugar substitutes between main meals. The control group was observed without intervention during the study procedures. At the end of study, for ethical reason, all subjects of control group also had professional dental cleaning and oral hygiene education.

Dental plaque analysis

Supragingival plaque was collected from the buccal and lingual gingival crevices of either the upper right or left first molar teeth by swabbing back and forth using a sterile excavator. All specimens were placed into a cryotube with 1 ml of reduced transport fluid and stored at -20°C until culturing process. The specimens were subsequently transferred into nutrient broth, after dispersed by vortexing for 10 seconds prior to incubate in BHI-B medium at 37°C within 24 hours of collection. Bacterial growth was measured in an optical density of suspension cells using spectrophotometer to determine microbial cell mass parameter or number of colony forming unit (cfu). Representative bacterial colonies from each plate were gram stained and microscopy identified in terms of the characteristic appearance.[13]

Asthma score

The subjects' parents or legal guardians completed a detailed questionnaire regarding demographic and asthma history with questions concerning triggers, medication use, and other asthma-related variables. Assessment of asthma symptoms such as cough, wheeze, and wake up during the night, and daily activity based on a 4-point scale, which ranged from no symptoms to severe difficulty, were made during seven days of dental treatment.[14] Parents or responsible persons were given instruction to facilitate allergen avoidance of various potentially allergenic foods, house dust and pet control.

Lung function test

A bronchodilator challenge was performed at first and second visit to detect reversible airway obstruction (asthma), carried out by the principal investigator, using a portable microprocessor-based electronic spirometer that displayed a spirogram as well as the lung function reading. It would automatically generate subject's age, sex, race, height and weight into the predicted normal lung-function values based on the regression equations of Polgar. The spirometer, which met American Thoracic Society (ATS) standard, was calibrated each day of the study to maintain quality control. The lung function test was conducted between 8 to 9 a.m. to standardize and account for daily variation in asthmatic response. To ascertain reproducibility of spirometry results, at least three acceptable spirograms must be obtained. When a minimum of three tests were performed, the spirometer selected the best reading and calculated the percentage of the predicted lung function value for that reading.[15]

Statistical analysis

The paired t-test was used to compare the number of colonization and detection percentages of bacterial

morphology composition in both treatment and control groups before and after study procedures for the bacterial profiles changes and for asthma score, FEV1 reversibility, and blood eosinophil count. Influence of dental plaque control and bacterial profiles changes on the respiratory quality were evaluated by correlation analysis. The level of statistical significance was set at P value < 0.05 with and 95% confidence interval. We used SPSS package (version 14.0 for Window) for data analysis.

Results

Of the randomly selected 40 subjects, a total of 36 mild asthmatic children who qualified for the study were screened into treatment and control group. Each group consisted of 18 subjects with no differences in demographic characteristic, clinical history and initial laboratory results between two groups (**Table 1**). Ten boys and eight girls who received periodontal treatment for seven days had mean age 9.2 (SD 2.3) years and average body mass index (BMI) 15.2 (SD 2.3) kg/m^2, while 9 boys and 9 girls in the control group had mean age 8.9 (SD 2.3) years with BMI 15.1 (SD 2.3) kg/m^2. Measurement of dental plaque index, asthma score, lung function, and blood eosinophil concentration for baseline data also revealed no differences between the two groups of children.

Table 1. Demographic characteristics of the participants

Variables	Treatment group (n = 18)	Control group (n =18)
Duration of asthma:		
3-6 months	4	3
6-12 months	2	2
> 12 months	12	13
Allergy risk (trace card):		
10%	3	3
20-30%	1	1
20-40%	8	9
60-80%	6	5
Positive skin test result:		
House dust allergy	16	16
Food allergy	18	18
Pet allergy	10	9
Asthma triggers:		
Allergen exposure	15	16
Cold temperature	5	4
Emotional stress	3	4
Physical exercise	4	4
Night	7	8
Current therapy:		
Inhaled β2-agonist	4	3
Ingested β2-agonist	5	4
Inhaled corticosteroid	0	0
Ingested corticosteroid	2	1
Other asthma drug	1	1

During the seventh day study period, subjects who were given oral health education reported an increased in tooth brushing frequency into three times daily (P<0.001), but none of participants in control group reported any changes in oral hygiene behavior from their previous habit. After periodontal control, our study subjects in the treatment group demonstrated significant reduction of dental plaque index as 2.4 (SD 0.5) (P<0.001), approximately 86% of its original level before dental plaque control was initiated. Only two children in control group showed plaque index reduction, without substantial change in total analysis.

Marked differences in bacterial profiles as response to dental plaque removal, represented in amount of colonization and morphological composition of dental plaque micro flora, were observed among treatment group. Significant reduction with mean 1.7 (SD 1.4) x 10^8 cfu/ml (P<0.001) occurred in number of microbial colony forming units. The number of bacterial colonization was 40.7% lower when compared to its initial level. Reduction in bacterial number was not achieved in the control group (**Table 2**).

Microbiological analysis before the start of dental treatment revealed detectable growth of bacteria on all plaque culture from both treatment and control group. Compare with control and pre-treatment values, significant reduction was seen in gram negative bacilli colonization (P < 0.0001) on dental plaque swabs of 10 (55.6 %) subjects. Since no dental intervention was introduced to control group, initial and follow up plaque culture showed almost identical results (**Table 2**).

Complexes of bacteria may present on each dental plaque culture. However, usually there was only one that was considered as a major microorganism. Initially, distribution of dominant bacterial morphology was found to be similar between the two groups. At refrain analysis, we identified different composition between treatment and control group, especially for gram negative

Table 2. Bacterial profiles on dental plaque culture

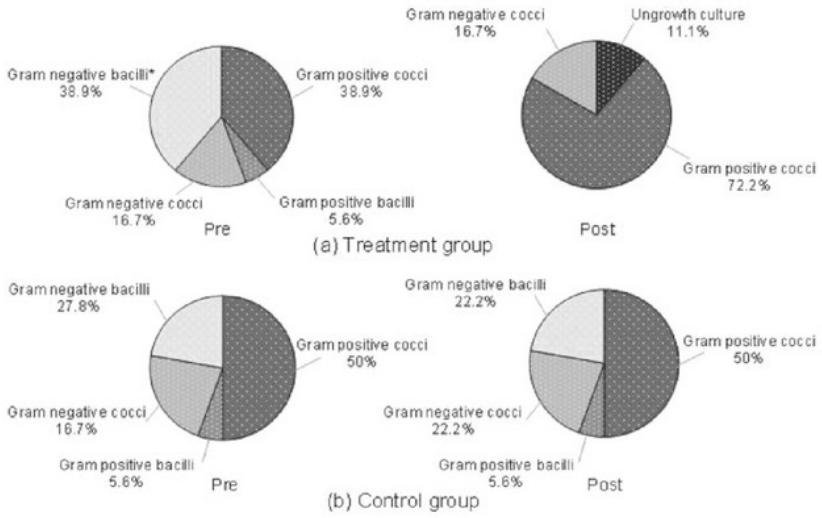
Parameters	Treatment group (n = 18)		Control group (n = 18)	
	Pre	Post	Pre	Post
Bacterial number (x 108 cfu/ml, mean ± SD)	5.0 ± 2.0	3.2 ± 2.1*	5.3 ± 2.2	5.0 ± 2.2
Colonized bacterial morphology				
- Gram positive cocci	14	13	14	14
- Gram positive bacilli	3	3	4	4
- Gram negative cocci	9	3	8	6
- Gram negative bacilli	12	2*	13	10

*P<0.01 compared with controls and pre-treatment values

bacilli (P=0.011), which had been eliminated at the end of study period. There were also two ingrowths cultures in treatment group taken after seven days of periodontal treatment (**Figure 1**).

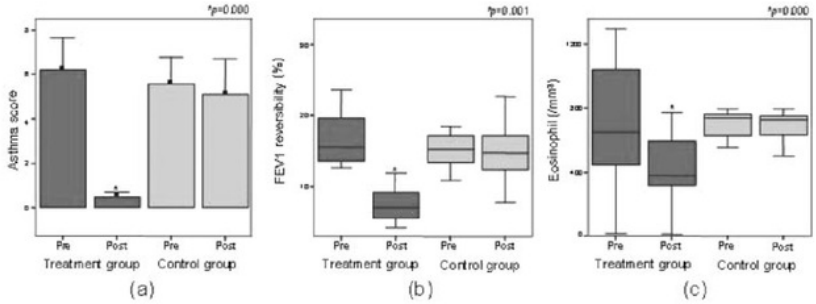
During the study period, none of our participants was reported to have an asthma exacerbation. Assessment of respiratory quality was based on asthma score, lung function test and blood eosinophil. Daily asthma symptoms were recorded by each participant, including cough, wheeze, sleep disturbance or wake up during night, and any limitation of their activity. The treatment group experienced clinically significant improvement over time, as shown by a decrease 5.7 (SD 3.1) (P<0.001) of asthma symptoms score. At seven day run-in dental plaque control, all subjects in treatment group demonstrated a significant amelioration of bronchial hyper reactivity (BHR), monitored by decrease in FEV1 reversibility with mean change 11.9 (SD 3.5)% (P<0.001) from baseline. Blood eosinophil as indirect measurement of airway inflammation also presented significant reduction of 292.7 (248.6) /mm^3 (P < 0.0001) **(Figure 2).**

Further correlation analysis was performed to estimate the magnitude of association between changes of bacterial profiles in subject with dental plaque control and the subsequent improvement in respiratory quality variables. Total bacterial number was significantly decreased after periodontal treatment,



*P<0.01 compared with controls and pre-treatment values

Figure 1. Distribution of dominant bacterial morphology



*P<0.01 compared with controls and pre-treatment values

Figure 2. Measurement of respiratory quality variables

but the changes did not have strong relationship (r<0.4; P<0.05) with asthma score and lung function. The number of colonization was not correlated with blood eosinophil. Significant moderate association (r>0.4; P<0.05) was seen between respiratory quality variables and a reduced rate of gram negative bacilli. It could be interpreted that improvement of respiratory quality in mild asthmatic children was related to eradication of gram negative bacilli due to dental plaque removal (**Figure 3**).

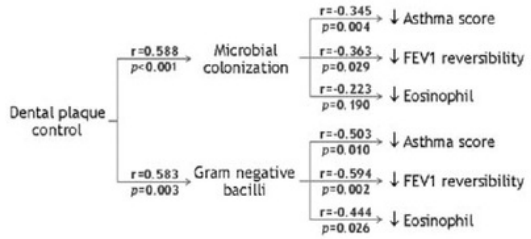


Figure 3. Scheme of correlation between periodontal treatment and respiratory quality

Discussion

This study shows the benefit of dental plaque control on respiratory quality of mild asthmatic children. We found that clinical symptoms, lung functions, and immunological parameters, as respectively mentioned in asthma score, FEV1 reversibility, and blood eosinophil, were experiencing a significant amelioration after periodontal treatment, consisted of professional periodontal treatment followed by individual oral health care, compared to the control group. None of those asthma severity variables were previously mentioned in the literature to have association with oral hygiene behavior. Our purpose was to determine any component in dental plaque, which may cause a decrease in respiratory quality, thus this finding would add another prevention approach in asthma intervention.

The relationship between periodontal disease and several systemic diseases is well documented. The role of bacterial infections in the development and progression of allergic inflammation provided important insight into oral infection to secondary systemic disease.[8] Studies and clinical experience indicate that periodontal disease may have an impact on infective endocarditic, atherosclerosis, myocardial infarction, diabetes mellitus, preterm delivery of low birth weight infants, and bacterial pneumonia. Microbiologic and immunological findings have also lent credence to the concept that periodontium may serve as a reservoir of bacteria, bacterial products, and inflammatory and immune mediators which can interact with other organ systems remote from the oral cavity.[16-18]

There is only very little information available concerning oral health and of asthma in children, therefore it continues to be an active area of investigation. A systematic review by Scannapieco and colleagues[19] concluded that oral colonization fostered by poor oral hygiene might promote a higher concentration of oral pathogens in the dental plaque and saliva, at the capable amounts of causing chronic respiratory disease. Katancik and colleagues[20] conducted a cross sectional study which revealed that participant with normal pulmonary function

had significantly better gingival index and loss of attachment scores than those with airway obstruction, based on the FEV1/FVC ratio and the percent of predicted FEV1, but it cannot provide direct inference of cause and effect between periodontal disease and airway obstruction. However, there are still no sufficient evidence to claim a causal association between oral infection and asthma.

The association between early childhood lower respiratory infection and subsequent developments of asthma were elegantly assessed in a birth cohort study by Illi and colleagues.[21] More recently, Bisgaard and colleagues propose an alternative explanation, that bacterial colonization of the airways may induce neutrophilic inflammation and thereby cause asthma.[22] In an accompanying editorial of the issue, Von Mutius said the most likely explanation is that early bacterial colonization reflects an innate immune defect in children at risk of atopy that appears to promote the development of asthma.[23]

Nowadays, the knowledge that asthma is an inflammatory disorder has become fundamental. Inflammation leads to an increased bronchial hyperreactivity which in turn viewed as variable airflow obstruction follows exposure to inducers such as allergens, viruses, exercise, or nonspecific irritant inhalation. Bronchial hyperreactivity has been ascribed to the effect of interaction of inflammatory cells, mediators, neuropeptides, hormones and the sympathetic to parasympathetic relations acting upon target cells such as epithelial, endothelial, bronchial smooth muscles and glandular cells, implying the concept of neurogenic inflammation.[24] A cascade of immunological stimulation in which mast cells and eosinophil play pivotal roles, and considered to be the key event in the onset of bronchial hyperreactivity, especially in school-age children.[25]

In common practical setting, as it is possible for asthma patient to be relatively asymptomatic during dental treatment, routine dental plaque controls do not appear to help predict outcome of patients with asthma. Therefore we performed spirometry testing to identify the effect of periodontal treatment on pulmonary function. One of the benefits of spirometry testing is that it can detect abnormalities in lung function even when no sign or symptom of disease is evident (Level I to II).[15] Reliable assessment of airway inflammation is also important because it provides an insight into the immunopathology of asthma, and also may lead to a more definitive assessment of the efficacy of asthma medication than asthma symptoms and lung function parameters. The degree of eosinophilia is proportional to the severity of asthma, as measured by clinical grading or pulmonary function.[26] Most of participants showed high concentration of blood eosinophil, implying that atopic asthma is associated with ongoing inflammation.

To the best of our knowledge, this study was the first to evaluate pulmonary inflammatory response to dental treatment in children with asthma. Although there are some conflicting findings and potential problem regarding uncontrolled underlying risk factor, its contribution to reliable clinical prediction was unclear. Most of the subjects in this study indicate a negative correlation between dental plaque control and asthma. On the basis of clinical impression suggesting that the quality of respiration in mild asthmatic children significantly improved along with the decreased amount of dental plaque colonization and changes in bacterial profiles followed periodontal treatment, we hypothesized that dental plaque as microbial reservoir may play a role in the etiology of allergic inflammation.

Although chronic inflammation due to infectious exposures in asthma has now been clearly established,[27] inhaled agents like bacterial dental plaque is less clearly defined. Dental plaque formation by periodontal pathogens initiates oral infection which leads to inflammation of periodontal tissues.[8,28] There are three mechanisms or pathways linking oral infection to secondary systemic disease: 1) Metastatic spread of infection from the oral cavity as a result of transient bacteremia; 2) Metastatic injury from the effects of circulating oral microbial toxin; 3) Metastatic inflammation caused by immunological injury induced by oral microorganisms. Ensuing local inflammation processes produce micro-ulcerations, which are conducive for transient bacteremia.[16,24] Moreover, bacteria release a variety of biologically active molecules, including lipopolysaccharides (endotoxins), chemotactic peptides, proteins toxins, and organic acids that may then enter the systemic circulation. These products can trigger the host inflammatory response and elevate serum concentration of acute-phase reactants and inflammatory mediators.[28,29] Increased

levels of circulating inflammatory mediators released by dental plaque biofilm is thought to contribute to the inflammatory processes leading to asthma.

Regular cleaning of bacterial plaque is essential for the prevention and ultimately effective to slow the progression of oral inflammation. With normal oral health and dental care, only small numbers of mostly facultative bacterial species gain hematogenous access to the blood stream or directly aspirate into the lung.[7,16] In contrast, poor oral hygiene increases the plaque load of pathogenic bacteria, especially supragingival, could increase 2-10 folds and thus possibly leading to a higher prevalence and magnitude of bacteremia.[18] The plaque removal significantly reduced plaque accumulation as well as noticeably fold-fall of dental plaque index in our treatment group. Similar with our result, in longitudinal study, Collins and colleagues[30] demonstrated improved gingival health associated with selective supragingival plaque with mechanical instrument combine with oral hygiene instruction.

In pediatric dentistry, periodic professional care has been considered the most effective method for plaque removal. Supragingival cleaning by dentist, included mechanical debridement via scaling combine with 'assisted drainage method', induced the 'normal healthy' periodontal condition.[12,31] This 'normal healthy' state was maintained by regular tooth brushing and gargling of 0.1% hexetidine twice daily for seven days.[30] Healthy gingival plaque is initially aerobic gram positive cocci and rods. Most sub gingival plaque shift to facultative and strict anaerobic gram negative pathogens, but strict anaerobic gram positive microorganisms have also implied.[13] Goodson and colleagues reported microbiological impact of dental prophylaxis on 20 healthy subjects with significant reduction in bacterial amount without specifically change in their composition.[32] In the mean time, In our subjects, the total numbers of bacteria colonizing the teeth surfaces were successfully decreased and the proportion of bacterial profiles were also altered by controlling dental plaque.

Recent progress in identification and characterization of periodontal pathogens, as well as elucidation of potential systemic mechanisms of action of bacterial products and inflammatory cytokines,[33] have opened the way for a more realistic assessment of the systemic importance of periodontal disease. Molecular analysis using PCR techniques by means of DNA probe sequences and immunological reagents using either an enzyme-linked immunosorbent assay or an indirect immunofluorescence assay has the advantage of being rapid, more sensitive, specific and relatively inexpensive,[13] but these procedures remain unavailable in our microbiology laboratory today. Conventional culture technique was used to determine the composition of bacteria involved in this study. Unfortunately, anaerobic cultures of oral microorganisms have serious limitation considering the appropriate process becomes so labor-intensive and prohibitively expensive. There are 30% strains that also uncultivable because of mixed community networks in dental plaque biofilm, therefore we cannot identify the species using this method.[13] Then, we decided to characterize the bacteria morphology by direct microscopic identification stained with gram method.

Microbial effects of dental treatment may be expected to produce beneficial effect on respiratory quality.[28] In our study, gram negative bacilli complex was significantly eradicated after intensive dental plaque removal. Significant reduced rates on dental plaque gram negative bacilli colonization after periodontal cleaning was found to be strongly related to an improvement of respiratory quality in asthmatic subjects. It is generally accepted that gram negative bacteria possess complex carbohydrates and proteins which released upon replication or destruction of the outer membrane constituent, called endotoxins or lipopolysaccharides (LPS).[34]

Explaining the biologic mechanism begins with a number of pathological manifestations resulting from LPS which may enhance inflammatory response mediated by the interaction between lipids. A component and the receptor of the innate immune system, LPS penetrate the periodontal tissue and subsequently recruit and activate immune cells to release inflammatory mediators such as cytokines, chemokines and prostaglandins.[33-35] In a separate set of experiment, dental plaque samples from localized aggressive periodontitis (LAgP) patients were found to induce a type 2 immune response triggering by prostaglandin (PGE2), leukotriene (LTB4), lipoxin (LXA4) and thereby promoting humoral immunity.[36] These mediators, although helpful in fighting insult to the body, can be harmful as well.

Inhaled LPS can exacerbate airway inflammation and airflow obstruction in allergic asthmatics. The first controlled exposure in humans to purified endotoxins by inhalation was reported by Cavagna and colleagues that showed that 80 ng of inhaled LPS was associated with bronchoconstriction. Allergic subjects are more sensitive than nonallergic subjects to the bronchoconstrictive properties of inhaled LPS. The amount of LPS influence immune development related to the severity of asthma both in atopic and non-atopic subjects.[37] In allergic mild asthmatics, exposure to air containing a low level of endotoxins (250 ng/m^3) for four hours before bronchial challenge with allergen significantly increases both bronchial reactivity and antigen-induced airway eosinophilia.[35] Murakami[37] in 2006 provided a clue that LPS inhalation enhances Th2 responses as IL-5 expression by mast cell activation that unregulated eosinophil production, which exacerbate clinical features of asthma. In addition, there is some published data suggesting that prior allergen exposure significantly augments the inflammatory response to inhaled LPS. Endotoxins should be considered as an enhancing cofactor rather than inducing factor in asthma. Once the allergic phenotype is established, bacterial infection may be a synergistic factor on the amplitude of allergic response.[34] These are consistent with clinical evidence that microbial infection and exposure to endotoxins later in life occasionally exacerbates asthma.[38]

This study did not accurately define the specific nature of bacterial species that responsible for the effect. To discuss the possible ethiopathogenesis of our findings we try to extrapolate clinical studies that revealed the presence of principal bacterial species in plaque samples taken from healthy children aged between 5 and 9 years. Of these, the three most cited are *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Tannerella forsythensis* (formerly *Bacteroides forsythus*). The major periodontal pathogen, gram negative bacilli identified as *P.gingivalis*, has been isolated in approximately 50% of the dental plaque biofilm from these pre-pubertal children.[39-40] This circumstantial evidence eventually create a stronghold to an adaptation of *P.gingivalis* as the predominantly gram negative microorganisms found in our study.

Characteristic of *P. gingivalis* more closely resembles an opportunist or commensal than a exogenous oral pathogen, it bears low endotoxins activity that require TLR2 as predominant utilized commensally microbial pattern-recognition receptor.[41] Pulendran B and colleagues conducted a study analyzing the type of Ag-specific CD4+ Th and CD8+ T cell in response to LPS from *P.gingivalis*, which does not appear to require TLR4 for signaling. At low concentrations (<1 μg/ml), CD14 effectively transfers *P.gingivalis* LPS (PgLPS) to up regulate TLR2 and inhibit TLR4-mediated signaling. At higher concentrations (≥ 10 μg/ml), it induces strongly TLR2 expression and to a lesser extend TLR4.[42] This property is attributed mainly to the unique lipid A motif of PgLPS which contains unusual branches and relatively long fatty acids.[43]

Recognition of PgLPS was reported to have a preponderance of Th2 cytokines. PgLPS activated the DC subset to differentiate into Th2 cells responses, characterized by significant levels of IL-4, IL-5, IL-10 and IL-13, but lower levels of IFN-γ.[36-38,44] Jotwani and colleagues[45] studied the effect of LPS from *P.gingivalis* on CD14 molecules to induce the expression of IL-5, one of the typical Th2 cytokines that promotes the differentiation and survival of eosinophil. Therefore, it may explain that colonization with *P.gingivalis* play in eosinophilic inflammation in the causality of endotoxins in allergies and asthma. However, this proposed biologic model was beyond the scope of this study.

To substantiate the proposed etiopathogenesis concept, a prospective study in which the identification method of specific periodontal pathogens and the direct measurement of the resulting inflammatory mediator levels are made would be helpful in either approving or disapproving the hypothesis. If further research confirm that LPS of gram negative bacteria in dental plaque can activate the innate immune response to the subsequent development of asthma, then dental intervention should be recommend to prevent allergic reaction and asthma modulation.

The clinical implication of these findings is that the asthmatic children also need special attention in oral hygiene. Dental plaque control serves as an important component of healthy oral hygiene regimen. Cooperation between respirologist or allergy specialist and pediatric dentist team is also warranted. It seems justified to state that good oral health is important not only to prevent oral disease but also to maintain respiratory quality of asthmatic children.

We conclude that dental plaque control may

protect against microbial sensitization in asthmatic subjects. Asthma symptoms, lung function and airway inflammation in mild asthmatic children are significantly improved along with bacterial profiles changes following dental plaque control. A reduced number of bacterial colonization and an elimination of gram negative bacilli due to periodontal treatment are associated with the improvement of respiratory quality.

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