

## 論文要旨等報告書

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学位論文題名	CARIES, CARIES RISK, LIFE STYLE AND THEIR RELATION WITH MICROBIAL SCREENING IN JAPANESE CHILDREN (日本人小児におけるカリエスリスク、齲蝕と生活習慣ならびに細菌学的スクリーニングの関連)

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### 学位論文内容の要旨

#### (INTRODUCTION)

A key component of any preventive program is to assess a person's risk of developing a disease. Children are important subjects for dental health education because in this period, the primary teeth erupt, they are at higher risk for early bacterial colonization, and they begin to form their lifelong dental behaviors. A child's mother plays a vital role in raising and transferring health-related habits to her children. Assessing a child's risk of developing caries is a vital part of caries management. A comprehensive caries assessment should consider factors such as past and current caries experience, presence of cariogenic bacteria, and life styles. Children who experienced infection by mutans streptococci early in their lives had greater risk of developing caries than those who were infected later. These bacteria are acidogenic, aciduric and produce specific intra and extra polysaccharides that facilitate microbial adherence on the teeth. Their amount and virulence, together with patient's age at which these bacteria were contracted, are important factors in determining the course of dental caries. The genetic diversity of these cariogenic bacteria is still controversial; *S. mutans* has been shown to be more prevalent while *S. sobrinus* is more closely related with high caries activity and seldom found without *S. mutans*. However, it is difficult to identify caries risk of children on the basis of oral examination only. Many bacteriological caries activity test have been developed to avert this difficulty. Theoretically, dental plaque seems to be a particularly appropriate method because the tooth surface constitutes the natural habitat of *S. mutans*. The Cariostat test is a caries risk assessment tool that could predict children's caries risk. The Cariostat medium contains 20% of sucrose and two kinds of pH indicator to show visually the pH decrease caused by micro organisms in the patient's plaque sample. Moreover, a longitudinal study is more appropriate for following the changes of dental progression, oral hygiene of children, and looking for predictors of caries.

#### (MATERIALS AND METHODS)

A total of 283 children were selected based on these criteria: they had dental examination, had filled out the questionnaire and had participated in Cariostat microbial sampling. The dental examinations were carried out by the same dentist with a dental mirror and explorer under natural light. The same dentist also collected plaque samples from the maxillary buccal cervical surfaces using sterile cotton-tipped applicators. A dental hygienist then incubated the Cariostat medium at 37° C for 48 hours. In the present study, the Cariostat result were grouped according to low risk group (CAT 0, 0.5, 1.0 and 1.5) and high risk group (CAT 2.0, 2.5 and 3.0). A structured questionnaire was completed by the mother of the children at each screening to evaluate which factors regarding the children's life style made them susceptible to caries. The chi-square and student t-test were used to analyze significant ranking of children's lifestyles and how children's life styles and caries risk at the 1.5-year-old period would influence children's average caries at the 2.5-, and 3.5- year period. In a different study of 499 children, one hundred children were randomly selected from low and high caries risk children. Totally, 200 children were selected for microbial screening. Simple

random sampling was performed using three-colored dices thrown at the same time. Microbial screening of their Cariostat-inoculated plaque sample was done for detecting the presence of *S. mutans* and *S. sobrinus* on the basis of caries activity. DNA extraction was done following a protocol for Gram positive bacteria. Bacterial DNA detection was initially done through a PCR technique using universal primers to check for the presence of bacteria. The PCR reaction mixture (20 µl) contained of 2.0 µl of 10X PCR buffer, 1.6 µl of dNTP mixture, and 0.1µl of Taq DNA polymerase (*Takara Taq™*), 5.9 µl of distilled Water (*GIBCO™*), 10 µl template solutions and 2 µl each of the primer pairs. Besides the samples, purified genomic DNA from *S. mutans* ATCC 25175 and *S. sobrinus* ATCC 33478 were used as positive controls in each experiment. The PCR amplification was performed under thermal conditions specific for both bacteria. The PCR products were analyzed by electrophoresis on a 1% agarose gel in Tris-acetate-EDTA running buffer (pH 8.0). To estimate the size of the PCR products, a 100-bp DNA ladder and Ø X174 RF DNA/Hae III fragment were included in each gel as markers. After staining with 2% ethidium bromide, the newly synthesized DNA fragment was visualized under ultraviolet light. PCR products were verified at least two times under the same conditions, in accordance to the presence or absence of DNA bands.

#### (RESULTS)

- a. Cariostat score during the early childhood period showed the current oral condition and succeeded in predicting oral condition at a later age.
- b. Children's life styles affect caries risk.
- c. Cariostat test is useful in assessing caries increment.
- d. The presence of *S. sobrinus* was more closely related with high caries activity and high df numbers.

#### (DISCUSSION)

Knowledge about all of the risk factors in children is important for determining the optimal period for prevention and interceptive treatment. Caries experience and caries activity in children tend to increase as their age increases. Before a carious lesion actually develops, caries risk assessment is recommended. For life style at the 1.5-year-old period, a strong difference of the mean number of carious teeth was found between children with and without pre chewing habit. This negative parental behavior plays a role in early vertical transmission by salivary contact. The effect of breastfeeding to children's caries occurrence is still controversial. A few specific case studies have linked prolonged ad libitum and nocturnal breastfeeding to early childhood caries. For life style at the 2.5-year-old period, children with high sugar intake were clearly associated with an increase in caries at the 3.5-year-old period. Children with irregular snacking had a significant influence on children's decay rate. Initial PCR using universal primers showed a band indicating the existence of some bacteria throughout the whole plaque samples. It has been suggested that these bacteria are generally established in the oral cavity of children before 3 years old. Our PCR results show that these cariogenic bacteria are more prevalent in a child who has high caries risk than low caries risk, and agrees with several previous studies. This finding also associated the presence of cariogenic bacteria in children with high number of decay. *S. sobrinus* and the presence of both *S. mutans* and *S. sobrinus* were more likely detected in children in the high caries risk group and had high number of df. *S. mutans* is undoubtedly one of the most acidogenic of the species found within the dental plaque, being capable of producing acid from fermentable carbohydrates at a higher rate and over a much greater pH range than most other streptococci. Moreover, the presence of *S. sobrinus* was more closely related with high caries risk activity and high df numbers. Based on these studies, we summarize that: Cariostat score at early childhood not only show the present oral condition but also could predict their oral condition at a later age. Children's life styles change according their age and can potentially affect children caries status. The Cariostat test is useful in assessment and predicts children caries risk. Our microbial screening result show that the presence of *Streptococcus sobrinus* is more closely related with high caries activity and high df number of children.

## 論文審査結果の要旨

齲蝕罹患の危険性を齲蝕罹患または進行拡大する前に分析し予防することは、とくにその変化の著しい小児においては重要なことである。齲蝕は、養育環境に大きく左右される疾患であり、小児の口腔内の状態、齲蝕罹患の危険性ならびに生活スタイルにまで言及して予防を行う必要がある。

本研究は1.5歳, 2.5歳, 3.5歳児健診時に保健センターで行われた口腔内健診、齲蝕活動性試験カリオスタットならびにアンケート調査の結果から小児期の齲蝕予防の要点を解析し、さらに2.5歳児の口腔内から分離される*S. mutans*、*S. sobrinus*の存在と口腔内の状態の関係をdf歯数と齲蝕活性度によって評価したものであり、次のような結論を得た。

- 1) 1.5歳時の(Low risk: カリオスタットスコア、1.5以下とHigh risk: カリオスタットスコア、2.0)スコアをもとに被験者が2.5歳ならびに3.5歳児になった時のdf歯数と比較したところ両年齢時の平均df歯数は有意にHigh risk児のそれが多かった。さらに2.5歳時のカリオスタットスコアをもとに分析すると3.5歳時におけるdf歯数で同様の結果を得た。
- 2) 齲蝕活性度に有意に関与するライフスタイルは、噛み与え、養育者、授乳の継続、仕上げ磨き、間食の回数と遊びながら間食を食べる、近所からお菓子をもらうなどであった。
- 3) 1.5歳時と2歳時の生活スタイルは、それ以降の年齢の齲蝕活動性にも影響を与えた。
- 4) Low riskに比べHigh risk groupでは、*S. sobrinus*あるいは*S. mutans*と*S. sobrinus*が同時に高い確率で検出され、df歯数も大であった。これらの知見は、小児の齲蝕予防に役立ち歯科保健指導にエビデンスを与えるものである。

よって本論文は博士(歯学)の学位授与に値すると判定した。