

Practical application of Cari Screen test and ecological balance hypothesis*

Praktyczne zastosowanie testu Cari Screen i hipoteza o równowadze ekologicznej*

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Abstract

The aim of the study was to determine the oral health status, and to assess the efficacy of Cari Screen test to estimate the risk of caries in 3-year-old children. The ecological balance hypothesis suggests that caries risk increases cellular activity of dental plaque bacteria. The observations were conducted on a group of 30 randomly selected 3-year-old children. The researcher determined the value of dmft and the presence of tooth decay and dental plaque. Examinations of oral cavity were completed by Cari Screen (CS) test. Interpretation of results was based on a simple scale: 0-1500 for low activity of bacteria, with scores above 1500 indicating their high activity. The incidence of caries reached 60% in the 3-year-olds, and the average dmft number was found to be 3.3. During the Cari Screen examination difficulties in cooperation with such young patients were encountered. Preliminary analysis of the results of CS test indicates a higher ATP-driven bioluminescence of bacteria in children with active caries, however to formulate clear conclusions further research is required. The level of dental caries among examined children is alarmingly high. Cari Screen test used to examine 3-year-old children proved difficult to perform. This led to a modification of the procedure, which allowed correct use of this tool in youngest patients. New tools, which fit into more

Streszczenie

Celem pracy było ustalenie stanu zdrowia jamy ustnej i ocena skuteczność testu Cari Screen dla oszacowania ryzyka próchnicy w populacji trzylatków. Zgodnie z hipotezą o równowadze ekologicznej ryzyko próchnicy podwyższa aktywność komórkową bakterii w płycie bakteryjnej. Obserwacje przeprowadzono na grupie trzydziestu losowo wybranych trzylatków. Prowadzący badanie ustalił wartość liczby puwz, obecność próchnicy i płytki bakteryjnej. Badanie jamy ustnej przeprowadzono przy użyciu Cari Screen (CS). Interpretację wyników oparto na prostej skali: 0-1500 dla niskiej aktywności bakteryjnej a wyniki powyżej tej wartości sugerowały wyższą aktywność. Występowanie próchnicy osiągnęło 60% u trzylatków a średnie puwz wyniosło 3,3. Podczas badania napotkano na trudności w jego przeprowadzeniu w tej grupie wiekowej. Wstępna analiza wyników badania CS wskazuje wyższą bioluminescencję bakterii u dzieci z aktywną próchnicą jednakże konieczne są dalsze badania dla wyciągnięcia wiarygodnych wniosków. Poziom próchnicy w badanej grupie dzieci jest alarmująco wysoki. Test Cari Screen okazał się trudny do przeprowadzenia w grupie trzylatków. Należało zmodyfikować procedurę, co umożliwiło wykorzystanie tego narzędzia u najmłodszych pacjentów. Nowe narzędzia, uwiarygodniające najnowsze hipotezy

KEYWORDS:

caries risk, ATP-driven bioluminescence, pediatric patient

HASŁA INDEKSOWE:

ryzyko próchnicy, bioluminescencja stymulowana trifosforanem adenozyiny, pacjent w wieku rozwojowym

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recent hypothesis of dental caries development, give an opportunity to study the activity of pathogens and to estimate the risk of this disease more accurately.

o rozwoju próchnicy dają możliwość badania aktywności patogenów i precyzyjniejszej oceny ryzyka tej choroby.

Introduction

Dental plaque constitutes a microbiological ecosystem in which non-mutans bacteria (mostly non-mutans *Streptococci* and *Actinomyces*) are the key microorganisms responsible for supporting dynamic stability on the tooth surface. Microbial acid adaptation and acid selection play a critical role for destabilizing the mineral homeostasis of the enamel. Once the acidic environment has been established, mutans streptococci and other aciduric bacteria may extend and promote lesion development. From now on, high proportions of mutans streptococci and other aciduric bacteria can be considered biomarkers of caries progression. Concentration of substrates controls the dynamics of the ecosystem. Carbohydrates are the primary fuel for dental plaque bacteria (ATP production and its equivalents) and high supply of sugars leads to proliferation of acidogenic and aciduric bacteria.^{1,2}

Studies of microbiological factors, carried out in the past several years, indicate a high level of complexity of formation and development of carious process. An assumption that oral cavity is an ecosystem, where many species cooperate or compete for the most favourable niches, explains many phenomena and becomes widely acclaimed model.^{3,4}

This model assumes the possibility of such an influence on the oral cavity ecosystem, which allows avoiding high risk of caries. Determining the activity of micro-organisms may become the basis for control of its condition. The Cari Screen is a new tool for describing the activity of bacteria.⁵

The aim of the study was to determine oral health status and to assess efficacy of Cari Screen test to estimate risk of caries in 3-year-old children. The ecological balance hypothesis suggests that risk of caries increases the cellular activity of dental plaque bacteria.⁶

Materials and methods

The whole project received acceptance of the Bioethics Committee. The study was conducted on a group of 30 randomly selected 3-year-old children living in a small town environment. Moreover, the results were analysed regarding the presence of caries and hygiene level. Carious decay was assessed according to epidemiological standards of WHO using the dmft index. Hygiene was determined as insufficient if in at least one sextant (55-54, 53-63, 64-65, 74-75, 73-83, 84-85), dental plaque was visible to the naked eye. Absence of plaque was considered to be an indicator of effective teeth cleaning.

Observation of the metabolic activity of microorganisms was performed using Cari Screen method. This is a set consisting of a disposable test (Cari Test) and an electronic device that allows one to read immunofluorescent reaction.

Cari Screen measures ATP production originating from dental plaque cells. Adenosine triphosphate-driven (ATP-driven) bioluminescence can be quantified by measuring the release of visible light using the following reaction:^{1,2,5}

$$\text{ATP} + \text{Luciferin} + \text{O}_2 + \text{Luciferase} + \text{Mg}^{2+} \rightarrow \text{AMP} + \text{oxiluciferin} + \text{PPi} + \text{CO}_2 + \text{light (560nm)}$$

A sample is obtained by rubbing the teeth with a sterile cotton swab (Cari Test), avoiding contact with gingiva and saliva from the lingual surfaces of mandibular incisors. Biological material acquired in this way was placed in a plastic tubule. After breaking a tip base, the swab was moistened with a Luciferin-reagent, thus enabling to read the ATP assay in the Cari Screen device. Biofilm activity was measured directly in Relative Light Units (0-9999). Interpretation was based on a simple scale:

0-1500 – low activity of bacteria

>1500 – high activity of bacteria.

High Cari Screen score coexisting with at least one other risk factor should be regarded as an indication

Table 1. Distribution of microbiological Cari Screen test results in the absence and presence of active caries in the group of twenty-four 3-year-old children

Characteristics of statistical distribution		dt = 0	
i – CariScreen score	≥0	≥ 1500	
k – group size	12	8	
min-max (Relative Light Units)	47-8138	5306-8138	
Median (Relative Light Units)	6137	7001	
Characteristics of statistical distribution		dt ≥ 1	
i – CariScreen score	≥ 0	≥ 1500	
k – group size	12	8	
min-max (Relative Light Units)	0-8676	3241-8676	
Median (Relative Light Units)	4525	6318	
		no significant statistical difference (p=0.89)	no significant statistical difference (p=0.51)

for increased need for prevention of tooth decay. In line with CAMBRA (Caries Management By Risk Assessment) recommendations, these factors include for example: low economic status, visible carious lesion, xerostomia, and poor hygiene.

Results

The results of CS test were summarized according to two criteria: the presence of active caries and incidence of dental plaque.

The examined children were divided into two equal groups. The first one, in which the dt-index equaled zero and the second one, where dt-index equaled or was greater than 1. In both, subgroups were selected comprising children whose results suggested an increased risk of tooth decay (exceeding 1500). In each of the 12-patient subgroups eight children were found with the score greater than 1500.

The same statistical analysis was performed among children with proper and insufficient oral hygiene. Median values and the arithmetic average of these subgroups (from $i \geq 0$) showed that there is a difference ($p=0.58$), but it is not statistically

significant. Similarly, the median of the subgroups of children where the CS-test equaled or was greater than 1500 did not vary significantly ($p=0.47$). CariScreen distribution of test results obtained in both subgroups, are presented in Tables 1 and 2.

Difficult cooperation with young children prevented proper examination in six cases. The main problem was large amount of saliva and uncontrollable movements of the tongue. Slight modification of the swab-procedure allowed correct execution of other tests.

Discussion

Caries risk assessment tools are an important diagnostic aid in the field of pediatric dentistry. American Academy of Pediatric Dentistry (AAPD) has recently promoted the use of microbiological testing as an adjunct measure in assessing dental caries. The etiology of caries is multifactorial, and caries risk predictors may be found among the oral microflora, diet, and host, all three of which are fundamental for caries development. The correlation between total oral bacteria and oral streptococci, including cariogenic streptococci,

Table 2. Distribution of microbiological Cari Screen test results in the absence and presence of active caries in the group of twenty-four 3-year-old children

Characteristics of statistical distribution	Proper hygiene	
i – CariScreen score	≥0	≥ 1500
k – group size	12	8
min-max (Relative Light Units)	47-8138	3272-8138
Median (Relative Light Units)	5542	6359
Characteristics of statistical distribution	Insufficient hygiene	
i – CariScreen score	≥ 0	≥ 1500
k – group size	12	8
min-max (Relative Light Units)	0-8676	3241-8676
Median (Relative Light Units)	6318	7266
	no significant statistical difference (p= 0.58)	no significant statistical difference (p=0.47)

infers that ATP-driven bioluminescence may also potentially serve as an indirect assessment determinant of dental caries risk.

Studies on activity of dental plaque bacteria in patients with braces have shown, that ATP-driven bioluminescence might serve as a useful tool in the rapid, chair-side quantification of bacterial load and in the assessment of oral hygiene during orthodontic treatment.

Clinical observations have shown that caries is associated with increases in proportions of acid-producing and acid-tolerating bacteria, especially mutans streptococci and lactobacilli. Strains of other species may contribute to enamel demineralization. Even bifidobacteria are recognized as potentially cariogenic. The glycolytic activity of a large number of oral streptococci has shown that some strains of *non-Mutans Streptococci (non-MS)* can still metabolize sugars to acid at a moderately low environmental pH rates comparable with those achieved by MS. However, patients whose diet involves regular consumptions of snacks, have greater proportions of MS and lactobacilli in dental plaque and a greater risk of caries.

Initial bacteria colonizing cleaned surface of a tooth are primarily streptococcal species: *S.mitis*, *S.oralis* and *S.sanguinis*. Only these three may account for more than half of entire early microflora. At that stage *Streptococcus mutans* represents only 2% or less of the initial streptococci population, irrespective of caries activity. As microflora ages it shifts from Streptococcus-dominant to Actinomyces-dominant. The proportion of MS in dental plaque which covers a white spot lesion is frequently higher than at clinically healthy surface, however, still rather low. MS constitute about one third of total flora in cavitated lesion in dentine tissue. Meanwhile, it has been shown that in the absence of MS, the initial lysis of enamel was initiated only by members of early microflora.

Mutans streptococci are the major pathogens of human dental caries because they are frequently isolated from a cavitated lesion, also MS are highly acidogenic and aciduric and produce water-insoluble glucan, which promotes bacteria adhesion. However, the relationship between MS and caries is not absolute. High proportion

of MS may remain on tooth surface without lesion development, and caries can develop in the absence of this species. It is suggested that another acidogenic and aciduric bacterial species are responsible for the initiation of caries.⁷

Many authors suggest that ecological balance concept should be extended. They pay attention to the balance of dental plaque (dynamic stability stage), which is dependent on all acidogenic and aciduric bacterial species. In this hypothesis, dental plaque is a dynamic microbial ecosystem in which non-mutans bacteria such a non-MS and *Actinomyces* are the key players for the maintenance of dynamic stability. Furthermore, when sugar is supplied frequently, or salivary flow is too scarce to neutralize the produced acids, the pH decrease in plaque may enhance the acidogenicity and acidurance of the non-mutans bacteria adaptively. Therefore, biodiversity may have a protective effect on the stability of biofilm.

Since all the bacteria that have been associated with caries belong to the normal microflora of the oral cavity, dental caries has been described as endogenous infection, so it may occur when members of the resident flora obtain a selective

advantage over other species whereby the homeostatic balance of the biofilm is disturbed.³

This model assumes the possibility of such an influence on the oral cavity ecosystem which allows avoiding the high risk of caries. Determining the activity of micro-organisms of plaque may become the basis for controlling its condition. New tool for describing the activity of bacteria is the Cari Screen test

Conclusion

The use of ATP-driven bioluminescence has broad implications in dentistry and can be used to ascertain the efficacy of interventional or preventive therapies. High level of cell activity could indicate a dynamic stability stage disorder.

Cari Screen assesses the metabolic activity of bacteria inhabiting teeth surfaces. The study attempted to evaluate the usefulness of this non-invasive method for testing 3-year-old children. Researchers encountered difficulties in obtaining biological material, however a simple solution was to put a cotton wool roll lingually next to lower incisors. This helped prevent the movements of the tongue and isolated the teeth from saliva.

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