

Criteria for Diagnostics and Microbiology of Carious Dentin in Deep Carious Lesions of Primary Teeth

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Критерии за диагностика и микробиология на кариозен дентин при дълбоки кариозни лезии на временни зъби

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Abstract.

Introduction

The issue with clinical diagnostics of the initial reversible inflammatory changes in the pulp has been discussed for a number of years. The subjective character of the pain, especially concerning children, poses an additional difficulty for the diagnostic process, which necessitates the use of visual clinical criteria that provide information for the inflammatory progression in the pulp.

The dental caries is a multifactorial disease. Although according to the latest definition of dental caries, according to which it is a behavioral disease with a bacterial component, the main role of the microorganisms remains primary.

Aim

To specify the diagnostic criteria in deep dentin carious lesions and asymptomatic closed pulpitis and to research the microbial profile of the carious dentin in different in severity carious lesions of primary teeth.

Materials and Methods

82 carious lesions have been included in the clinical study. The diagnostic criteria are: size, width and periphery of the carious lesions as well as color of the carious dentin.

The samples of the carious dentin were taken using a sterile excavator. The sowing is carried out in blood agar, in selective agar for lactobacilli and in Brain-Heart infusion broth. An identification of the isolates was carried out.

Results

2/3 of all carious lesions tested are diagnosed as asymptomatic closed pulpitis, and 1/3 as deep dental carious lesions.

*Most often, and in substantial amounts, *S. mutans* is isolated in the carious dentin, followed by *Lactobacillus* spp. A greater diversity of microorganisms could be observed in an asymptomatic closed pulpitis in comparison with the deep dentin carious lesions.*

Key words: diagnostics, carious lesion, *S. mutans*, *Lactobacillus* spp

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Резюме

Въведение

Проблемът с клиничната диагностика на началните обратими възпалителни промени в пулпата се дискутира от много години. Субективният характер на болката, особено когато се отнася за деца още повече затруднява диагностичния процес, което налага използването на визуални клиничните критерии, които да дават информация за напредването на възпалението в зъбната пулпа.

Зъбният кариес е многофакторно заболяване. Независимо, че според последното определение на зъбен кариес според, което това е поведенческо заболяване с бактериална компонента, основната роля на микроорганизмите си остава първостепенна.

Цел

Да се прецизират диагностичните критерии при дълбоки дентинови кариозни лезии и асимптоматичен затворен пулпит и да се изследва микробния профил на кариозен дентин при различни по тежест кариозни лезии на временни зъби.

Материал и методи

В клиничното проучване бяха включени 82 кариозни лезии. Критериите са диагностика са: големина, ширина и периферия на кариозните лезии, както и цвят на кариозния дентин.

Пробите от кариозен дентин бяха взети с помощта на стерилен екскаватор, Посявката се извършва на кръвен агар, на селективен агар за лактобацили и на Brain-Heart infusion broth (бульон). Извършена беше идентификация на изолатите.

Резултати

От всички изследвани кариозни лезии 2/3 се диагностицират като асимптоматичен затворен пулпит и 1/3 като дълбоки дентинови кариозни лезии.

В кариозен дентин най-често и в най-големи количества се изолира *S. mutans*, следван от *Lactobacillus spp*. При асимптоматичен затворен пулпит се наблюдава по-голямо разнообразие от микроорганизми, сравнено с дълбоките дентинови кариозни лезии.

Ключови думи: диагностика, кариозна лезия, *S. mutans*, *Lactobacillus spp*

Introduction

The issue with the clinical diagnostics of the initial reversible inflammatory changes in the pulp has been discoursed for a number of years both internationally and nationally, but there are no concrete clinical parameters, which could provide correspond to the specific pathobiological processes in the pulp. The lack of possibility for tracing the main classical clinical symptoms of the inflammation necessitates the creation of a clinical classification of the pulpitis, where the emphasis is put on the pain as a symptom and the clinical characteristics of the carious lesion leading to the inflammation of the pulp [1, 2]. The subjective character of the pain, especially concerning children, poses an additional difficulty for the diagnostic process, which necessitates the use, with a maximum precision and in detail, of visual clinical criteria regarding size and location of the carious lesion that

indirectly provide information on the extent of the severity and inflammatory progression in the pulp [3]. Having taken into consideration the minimal invasive method, which took hold in the pediatric cariesology, detecting the early reversible stages of the pulp inflammation and differentiating them from the deep dental carious lesions is a monumental moment in dental diagnostics [4].

The tooth caries is a multifactorial disease, which develops through the activity of several main factors – enamel, microorganisms, carbohydrates, time and saliva. Although according to the latest definition of dental caries, according to which it is a behavioral disease with a bacterial component, the main role of the microorganisms remains primary [5, 6].

According to Phil Marsh's hypothesis, the tooth caries is due to an imbalance of the bacterial species which leads to a change in the local conditions of the environment as well as a dysbiosis..

The recurring cycles of low pH in sugars intake lead to the development of acidogenic and aciduric microorganisms with the acidogenic gram-positive types becoming dominant – *S. mutans* and *Lactobacillus spp* on the tooth surface [7, 8].

Aim

To specify the diagnostic criteria in deep dental carious lesions and asymptomatic closed pulpitis and to research the microbial profile of the carious dentin in different in severity carious lesions of primary teeth.

Tasks

Task 1 – Specification of the visual diagnostic criteria of deep dentin carious lesions and asymptomatic closed pulpitis in primary teeth;

Task 2 – Microbiological research of carious dentin of deep dentin carious lesions and asymptomatic closed pulpitis in primary teeth.

Materials and Methods

82 carious lesions, registered by ICDAS II (code 05/06) have been included in the clinical study of children aged between 4 and 7 years, 38 boys and 44 girls. Cavitated dentin carious lesions and asymptomatic closed pulpitis of primary teeth first and second molars have been selected – 16 carious lesions with occlusal localization and 66 carious lesions with approximal localization. The criteria, according to which the clinical diagnostic of the examined carious lesions has been carried out, are:

- Size of the examined carious lesions – affected adjacent tubercle;
- Width of the examined lesions in an vestibule-oral direction in approximal lesions and degree of fissure coverage in occlusal lesions;
- Periphery of the carious lesions – impaired transparency;
- Periphery of the carious lesions – transparent carious dentin;
- Color of the carious dentin as per the initial examination.

A microbiological study of carious dentin was carried out on 42 of the examined carious lesions –

20 cases of deep dentin carious lesions and 22 cases of asymptomatic closed pulpitis. The samples of the carious dentin were taken using a sterile excavator Koine. The examination material is placed in a sterile ependorf test tube with a transportation environment provided by the laboratory and is delivered up to 3-4 hours in the microbiology laboratory, whose director is prof. dr. R. Gergova, Medicinal Microbiology Department, MU-Sofia. The sowing is carried out in blood agar, in selective agar for lactobacilli and in Brain-Heart infusion broth, which are incubated for a period of 24-28 hours in a thermostat at 36°C in a CO2 atmosphere. If the growth of the solid medium is insufficient, the broth is re-sown after another enrichment of another petri. Clear cultures from the morphologically dubious colonies are isolated. An identification of the isolates was carried out.

Picture No 1 demonstrates the stroke method used for the sowing of the samples taken.



Picture No 1. Stroke Method Used for the Sowing of the Samples Taken

The data was statistically processed with the SPSS software (version 19, SPSS Inc., USA). A 95% interval of confidentiality was chosen as a level of significance ($p < 0.05$).

Results

Specification of the visual diagnostic criteria of deep dentin carious lesions and asymptomatic closed pulpitis in primary teeth

Table No 1 reveals the results of the five diagnostic criteria used in each of the examined carious lesions.

Table No 1. Diagnostic Criteria for Each of the Examined Carious Lesions

Diagnostic Criteria		Primary Molars		T-test
		N	% ± sp	
1. Size in an MD Direction	Does not reach the middle	28	34.15 ± 5.24	t = 4.28 p < 0.05
	Exceeds the middle	54	65.85 ± 5.24	
	Total	82	100%	
2. Width	Up to 2/3	28	34.15 ± 5.24	t = 4.28 p < 0.05
	> 2/3	54	65.85 ± 5.24	
	Total	82	100%	
3. Impaired Transparency	No	28	34.15 ± 5.24	t = 4.28 p < 0.05
	Yes	54	65.85 ± 5.24	
	Total	82	100%	
4. Transparent Carious Dentin	No	22	26.83 ± 4.89	t = 6.70 p < 0.05
	Yes	60	73.17 ± 4.89	
	Total	82	100%	
5. Color of the Carious Dentin	Light	54	65.85 ± 5.24	t = 4.28 p < 0.05
	Dark	28	34.15 ± 5.24	
	Total	82	100%	

It could be observed that, in relation to the size of the carious lesion, the cases suspected for asymptomatic closed pulpitis are almost twice as much as the deep dentin carious lesion ones. This tendency is also retained in the second and third diagnostic criteria – width of the examined carious lesion and impaired transparency. In 2/3 of the lesions in the enamel periphery there is a transparent dark coloring which is the result of the underlying carious dentin. This indicates for an advanced carious process with a clearly defined dentin cavitation. During the initial diagnostic of each carious lesion, the ones with a lighter carious dentin are predominant (65.85%), whereas the carious lesions with a darker dentin are almost twice less (34.15%) (p<0.05). This is understandable, taking into consideration that the carious process in pri-

mary teeth develops relatively rapidly, due to the lower mineralization of the dentin and the thinner layers of hard dental tissues.

Based on the followed clinical criteria, the examined cases were diagnosed as deep dentin carious lesion and asymptomatic closed pulpitis.

The results are shown in the following table.

Out of 82 examined carious lesions, 20 (24.39%) have been diagnosed as deep dental carious lesions, and 62 (75.61%) as asymptomatic closed pulpitis. The table demonstrates a statistically significant difference in favor of the asymptomatic closed pulpitis (p<0.05).

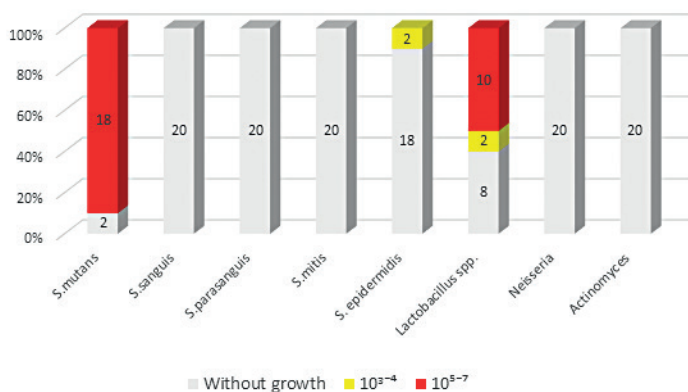
Microbiological research of carious dentin of deep dental carious lesions and asymptomatic closed pulpitis in primary teeth

Table No 2. Differential Diagnostic between Deep Dentin Carious Lesion and Asymptomatic Closed Pulpitis

Diagnosis	Total for all teeth	
	N	% ± sp
Deep Dentin Carious Lesion	20	24.39 ± 4.74
Asymptomatic Closed Pulpitis	62	75.61 ± 4.74
Total	82	100%
T-test	t = 7.64 p < 0.05	

The isolated microorganisms from the carious dentin in the examined clinical cases are shown in the subsequent graphs.

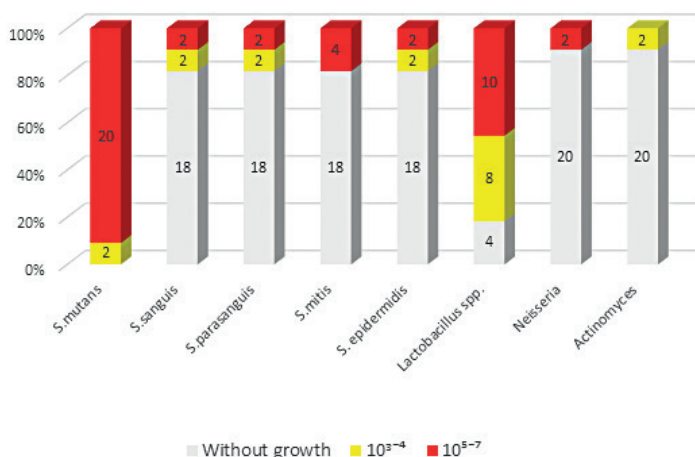
Graph No 1 reveals the frequency and amounts of cariogenic microorganisms from carious dentin samples in deep dentin carious lesions.



Graph No 1. Microorganisms in Caries Dentin in Deep Dentin Carious Lesion

In deep dentin carious lesions *S. mutans* is isolated in higher amounts, followed by *Lactobacillus spp.*, and *S. Epidermidis* – only in separate cases. It could be observed that no *S. sanguis*, *S. parasanguis*, *S. mitis*, *Neisseria*, *Actinomyces* are isolated.

The next graph demonstrates the amounts of the cariogenic microorganisms from carious dentin samples with asymptomatic closed pulpitis.



Graph No 2. Microorganisms in Carious Dentin with Asymptomatic Closed Pulpitis

In cases of asymptomatic closed pulpitis, the flora is more diverse. The most common and in

largest amounts microorganism is *S. mutans*, followed by *Lactobacillus spp.* and *S. mitis* – in 4 cases, and in separate cases with low amounts there are also *S. sanguis*, *S. parasanguis*, *S. epidermidis*, *Neisseria*. They define the greater variety of the cariogenic microflora in carious dentin in cases with asymptomatic closed pulpitis. The more complex microbial flora is identified in cases with lighter color of the carious dentin, typical for a faster carious process.

Discussion

The research reveals that the five clinical criteria (Table No 1) examined characterize the size and spread of the carious lesion in the dentin, its proximity to the adjacent pulp horns that is comparable to the degree of infection of the underlying coronary dental pulp of the examined primary molars. Everything is directly dependent with the degree of inflammation of the pulp. These clinical indicators, in conjunction with the subjective lack of spontaneous pain symptomatic, are sufficiently reliable criteria for making a differential diagnosis between deep dentin carious lesion and asymptomatic closed pulpitis in primary molars (Table No 2).

In accordance with the microbiological research carried out, it is clear that there is a difference in the frequency and the amounts of the isolated microorganisms. *S. mutans* is isolated in 100% of the asymptomatic closed pulpitis cases, and in 90% of the deep dentin caries cases (Graph No 2 and Graph No 1).

These results demonstrate that *S. mutans* is the main actor in the carious process.

From the streptococcaceae group *S. sanguis*, *S. parasanguis* and *S. mitis* are also isolated. These microorganisms are isolated in the carious dentin only in asymptomatic closed pulpitis (Graph No 2).

The second in frequency and amount microorganisms isolated are *Lactobacillus spp.* As op-

posed to *S. mutans*, they could be detected in half of the cases of deep dentin carious lesions and asymptomatic closed pulpitis (Graph No 1 and Graph No 2).

It could be observed that there is a greater variety of microorganisms, which are isolated in asymptomatic closed pulpitis in comparison with deep dentin carious lesions. *S. sanguis*, *S. parasanguis*, *S. mitis*, *Neisseria*, *Actinomyces* cannot be detected in deep dentin carious lesions (Graph No 1 and Graph No 2). This demonstrates that in carious lesions, which are in an earlier development stage, as are the deep dentin carious lesions, the microflora is less diverse in type and amounts of microorganisms. The more complex microbial flora could be seen in asymptomatic closed pulpitis cases which is characterized with lighter tones of the carious dentin. The color of the carious dentin is due to the more rapid development of the carious process. It could be concluded that the cariogenic microbial flora is related to the severity and speed of the process in the dentin.

A number of research studies regarding the microflora in the development of the carious lesions could be found in the literature. *Becker et al* compare the microorganisms detected in early childhood caries and in children having no carious lesions in primary teeth. They reach the conclusion that some types of *S. sanguinus* could be present without the existence of carious lesions, whereas the presence of *S. mutans*, *Veillonella spp*, *Actinomyces spp.*, *Bifidobacterium spp* и *Lactobacillus fermentum* are associated with the development of the carious process [9].

Aas et al examine the microorganisms in children without carious lesions and those with carious lesions, in different stages of development, in primary teeth and young permanent teeth. The samples taken are from intact enamel surface, white enamel carious lesions, cavitated dentin carious lesions as well as from deep dentin car-

ious lesions. The achieved result are the following: in 10% of the examined cases of permanent teeth of children *S. mutans* cannot be detected. In addition, *S. mutans* is not isolated from intact enamel surface nor from white enamel carious lesions. *S. mutans* is, however, isolated in dentin carious lesions [10].

In the development of the carious process a variety of microorganisms could be found, depending on the progression of the process. In white enamel lesions, as well as in dentin lesions, *S. parasanguinus* and *S. salivarius* are isolated in large amounts in both dentitions. The *Corynebacterium sp.* cl. AK153 microorganisms and *A. gerencseriaue* are isolated only in the primary dentition in large amounts, whereas in permanent dentition *Campylobacter gracilis* и *Selenomonas spp* cl. EY04 could be found. The microflora in the deep dentin carious lesions is represented by *S. mutans*, *Lactobacillus spp.*, *Propionibacterium spp.*, *Atopobium genemospecies C1* and *Bifidobacterium spp*. The authors determine that *S. mutans* has a dominating role in the development of the dentin carious lesions in the primary dentition [10]. Similar results are also achieved by Chhour et al and Corby et al who also conclude that *S. mutans* and *Lactobacillus spp* are dominating in the progression of the carious lesions. [11, 12].

Conclusions

1. The diagnostic criteria used by us, size, width, periphery of the carious lesion and color of the carious dentin, are sufficient in the differential diagnosis between long deep dentin carious lesion and asymptomatic closed pulpitis of primary teeth;

2. In a carious dentin in deep dentin carious lesion and asymptomatic closed pulpitis of primary teeth, most often and in greatest amounts *S. mutans* is isolated, followed by *Lactobacillus spp*;

3. In a carious dentin in asymptomatic closed pulpitis of primary teeth a greater varieties of mi-

croorganisms is observed, including *Lactobacillus spp*, *S. sanguis*, *S. parasanguis*, *S. mitis*, *Neisseria*, *Actinomyces*, which are always in combination with *S. mutans*

4. The lighter carious dentin, typical of a faster carious process, has a richer cariogenic microbial flora compared to the darker carious dentin in a slower carious process.

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