

ORAL HEALTH

DETERMINATION OF ULTRASTRUCTURAL CHANGES IN THE ENAMEL OF PRIMARY TEETH IN STARTING PHASES OF EARLY CHILDHOOD CARIES, WITH AND WITHOUT LOCAL FLUORIDE THERAPY

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Abstract

Early childhood caries is often found under the name connected with the manner of feeding nutrition of children such as “Baby bottle Syndrome” or “Baby bottle caries”. The aim of the study was to determine ultrastructural changes in the enamel of primary teeth in starting phases of early childhood caries, with and without local fluoride therapy, because it has of great influence in the preventive aspect of the disease. The investigation was done on laboratory tests on tree groups extracted primary teeth: one of 20 healthy mandibular incisors, as control group and two more groups of primary tested teeth: 10 maxillary incisors with initial lesion and 10 maxillary incisors with superficial form. The all dental samples were observed with Scanning Electron Microscope (SEM) and we made comparative analyzes in ultrastructural changes of enamel between untreated and treated teeth, and control group teeth. After that, we have analysis with SEM photo attachments. In some dental samples with initial lesion after local treatment we managed to get almost healthy enamel, i.e. complete remineralization process. Once again, we have confirmation of one of the many benefits of fluoride in dentistry which is long been known.

ОРАЛНО ЗДРАВЈЕ

ДЕТЕРМИНИРАЊЕ НА УЛТРАСТРУКТУРНИТЕ ПРОМЕНИ ВО ЕМАЈЛОТ НА МЛЕЧНИ ЗАБИ ВО ПОЧЕТНИ ФАЗИ НА КАРИЕС НА РАНО ДЕТСТВО, СО И БЕЗ ЛОКАЛНА ФЛУОРИДНА ТЕРАПИЈА

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Цитирање: Кокочева –Ивановска О. Детерминирање на ултраструктурните промени во емајлот на млечни заби во почетни фази на кариес на рано детство, со и без локална флуоридна терапија. Арх Ј Здравје 2023;15(2) 60:69. doi.org/10.3889/aph.2023.6099

Клучни зборови: млечни заби, емајл, реминерализација, флуориди, скенинг електронски микроскоп

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Печатарски права: ©2023 Олга Кокочева –Ивановска. Оваа статија е со отворен пристап дистрибуирана под условите на нелокализирана лиценца, која овозможува неограничена употреба, дистрибуција и репродукција на било кој медиум, доколку се цитираат оригиналниот(ите) автор(и) и изворот.

Конкурентски интереси: Авторот изјавува дека нема конкурентски интереси.

Извадок

Кариесот на раното детство се среќава под имиња поврзани со начинот на исхраната кај децата како “Синдром или Кариес предизвикан од исхрана со шише”. Целта на оваа студија беше да ги утврдиме ултраструктурните промени во емајлот на млечни заби во почетните фази на кариес на рано детство со или без локална флуоридна терапија, со големо влијание од превентивен аспект, за ова заболување. Во лабораториското испитување вклучивме три групи на екстрахирани млечни заби: една со 20 здрави млечни мандибуларни инцизиви, како контролна група и две испитувани групи млечни заби: 10 максиларни инцизиви со иницијални лезии и 10 максиларни инцизиви со суперфицијални лезии. Кај сите примероци на заби со Скенинг Електронски Микроскоп (SEM) вршевме компаративна анализа помеѓу ултраструктурните промени на нетретираниите и третираниите заби со локален флуориден третман и контролната група здрави заби. Резултатите покажаа дека кај некои забни примероци со иницијални лезии, добивме скоро здрав емајл, со речиси комплетна реминерализација, како резултат од реминерализирачкото дејство на локалната флуоридна терапија. Така уште еднаш се потврди еден од многуте претходно познати benefiti на флуоридите во детската стоматологија.

Introduction

Early Childhood Caries (ECC)¹ actually present one or few decayed lesions (cavitated or noncavitated), missing or filled tooth surface on primary teeth in children². ECC was often associated with the feeding and nutrition of children^{3,4} and named as “Baby bottle Syndrome” or Baby bottle caries”. Sugary liquid drinks (milk and juices) for longer period of time, with the presence of bacteria such as *Streptococcus mutans*, present very high risk^{5,6}.

ECC appears very early in babies from 6 months, it progress rapidly⁷ and often affects all four upper incisors.⁸ In the initial phase, ECC is presented like white demineralized enamel which quickly advances to decay around the gingival margin⁹. Carious lesions are spread on the labial or lingual surfaces of the teeth, or on both sides. The decayed tooth is with yellow or brown cavitated area.^{1,2,9}

The children disease is with multifactorial etiology and microbial investigations show presence of *Streptococcus Mutans* and *Lactobacillus*^{5,6}. Mother’s poor oral hygiene habit, dietary habit and activity which increase the possibility of saliva contact between mother and child are often mentioned as possibility of transmission risk *S. Mutans* in children aged 3-5 years old. Children with ECC have higher risk for developing caries in permanent dentition⁹, they regularly visit emergency services, have restricted feeding activities and poor oral health.

In the Republic of Macedonia, this type of caries is widely spread in pre-school children. In the central

area of the City of Skopje, children 18 to 42 months old, have 17, 9% of notified caries presence, which, according to the criteria of the World Health Organization, is estimated as high prevalence.¹⁰ In the developed countries as a result of effective and well timed implementation of the primary preventive measures, the Early Childhood Caries has relatively low prevalence of 3 %. In the undeveloped countries, because of lack of information on the adequate way of feeding and no solid oral hygiene^{5, 7} the prevalence of the Early Childhood Caries is up to 45%.

Clinical survey of Early Childhood Caries showed that this type of caries goes through several phases in its development¹⁰. Starting phase of development is the initial lesion (white spot lesion) and superficial form at the early childhood caries^{11, 12}.

Dental caries is a dynamic group of complex physical-chemical processes on the tooth surface that occur in vivo, with alternating periods of demineralization and remineralization and numerous inter reaction processes leading to imbalance and loss of minerals¹¹. Enamel demineralization is the process of dissolving calcium and phosphate ions from hydroxylapatite crystal, which then pass into the plaque and saliva. Initially, caries affects the hydroxyl apatite crystals and the hydroxyapatite demineralization process takes place.^{12,13} The early stage is the initial lesion, known as macula alba or white spots, which is reversible process¹⁴. At this stage the caries has not completely penetrated the enamel border. At the moment when it breaks, it spreads along it and then cavitation occurs due to cracking of

the enamel surface¹² and formed caries lesion (superficial form). Beneath the surface of the initial caries lesion or white spot there is a part with lost minerals just below the intact surface of the enamel. That part with fewer minerals is capable of a reversible remineralization process¹⁴. With remineralization, hydroxyl apatite increase and if fluoride is present in the medium, fluoride apathies will be formed¹⁵. Remineralization is the treatment for active initial unvaccinated carious lesion, allowing process reversibility or at least stopping progression to cavitation. From a preventive dentist practice, early detection (initial lesion) is of particular importance, because at this stage the caries process can be completely stopped or reversed¹⁵ (biological repair). But once the cavitation in the enamel has taken place, the caries process can be chronicized¹⁵ and to stop the further progradation

The aim of the research is to determine ultra-structural changes in the enamel substance in the starting phases of local fluoride therapy, because it has great influence in the preventive aspect of the disease.

Materials and methods

Clinical research

Taking into account the fact that the initial stages of early childhood caries, have an acute course and occur immediately after the eruption of teeth, in the clinical research, we included children of different sexes, aged 1.5 up to 3.5 years at the Clinic for Pediatric and Preventive Dentistry in Skopje, North Macedonia. We have made selection 117 children,

with a fully formed primary denture, in which by standard clinical examination we diagnosed early childhood caries in the early stages: initial lesion-white spot (macula alba) and superficial lesion.

Laboratory research

The research was performed on the group of children with a fully formed primary denture, and it is formed two basic groups:

- 60 children treated with local fluoride treatment
- 57 children followed, but did not undergo local fluoride treatment

From 57 children all of these, 31 children were diagnosed with an initial lesion, and 26 of them had a superficial lesion of the maxillary primary incisors.

Out of the same 57 children, after we ascertained the advanced physiological resorption in the observed teeth, we selected 10 of them with initial lesions and 10 with a superficial lesion of ECC, extracted one maxillary incisor.

Thus, in order to carry out further laboratory tests, we formed two groups of untreated samples:

I. Examined group of teeth:

- 10 extracted maxillary incisions with initial lesion-white spot (macula alba), and
- 10 extracted maxillary incisors with a superficial form of circulatory caries.

II. Tooth control group:

- 20 extracted healthy mandibular incisors

The above 60 children, covered with local fluoride therapy with p-p amino fluoride (once a week), were selected with equal attendance at the two initial stages of ECC, i.e.:

- ♦ 30 children with initial white spot lesion
- ♦ 30 children with superficial lesion

After completing the six-month local fluoride treatment, in further laboratory tests, we included:

III Examined group of treated samples with local fluoride therapy:

- ♦ 10 extracted maxillary incisions with initial lesion-white spot (macula alba), and
- ♦ 10 extracted maxillary incisions with a superficial lesion of ECC

The extracted teeth were kept in pure alcohol (96%) and before the

analysis, the teeth were dehydrated and covered by a thin layer of gold on the surface, with cathode dispersion technique. Investigation was made with the scanning electronic microscope (JSM 5300, SEM, JEOL, USA), and this procedure took place at the Institute for biomedical research of the Faculty of Medicine in Nis, Serbia. The enamel surface was observed and analyzed.

Results

1. SEM evaluation of ultra-structural enamel changes in untreated specimens of primary health incisors

- ♦ Ultrasound of healthy enamel tooth substance from the dental control group -mandibular primary incisors).

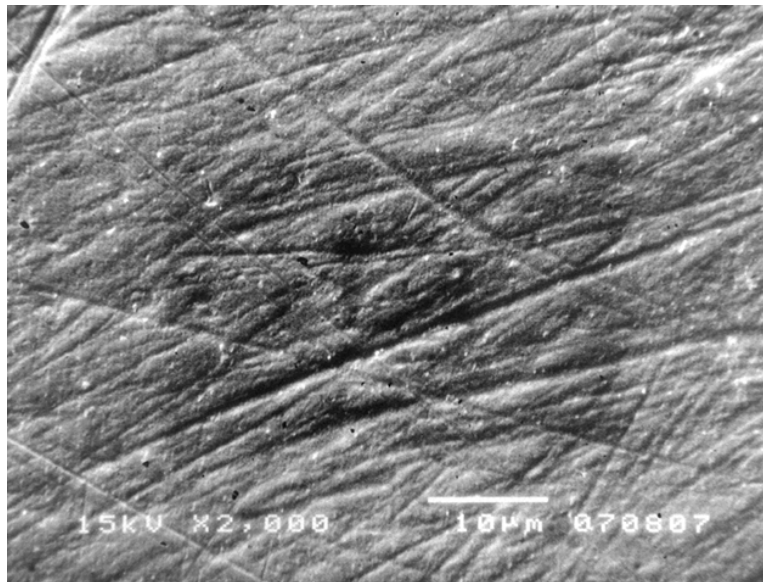


Figure. 1 Longitudinal section of a healthy enamel

On Fig. 1 is presented the longitudinal section of the outer surface of a healthy enamel. It is homogeneous, flat, with a less tooth brush traces wavy appearance (magnification 2000 times). The transverse lines

seen on the surface are visible traces of mechanical tooth brushing.

- ♦ Ultrasound changes in the untreated initial lesion (maxillary primary incisors)

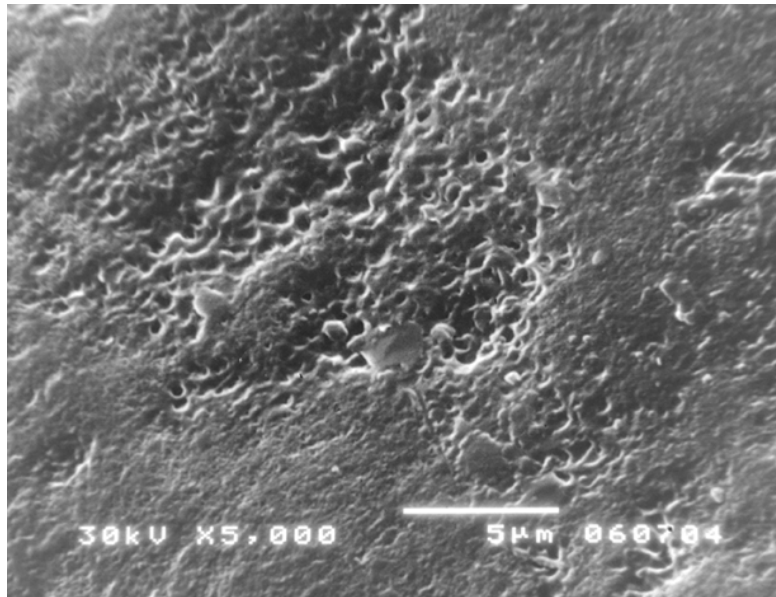


Figure. 2 Initial lesion of maxillary incisor

Fig. 2 shows an island of initial demineralization in the initial lesion and around it is retained and enamel cuticle of a healthy enamel (magnification 5000 times)

2. SEM evaluation in enamel ultrastructure of initial lesion after topical fluoride treatment

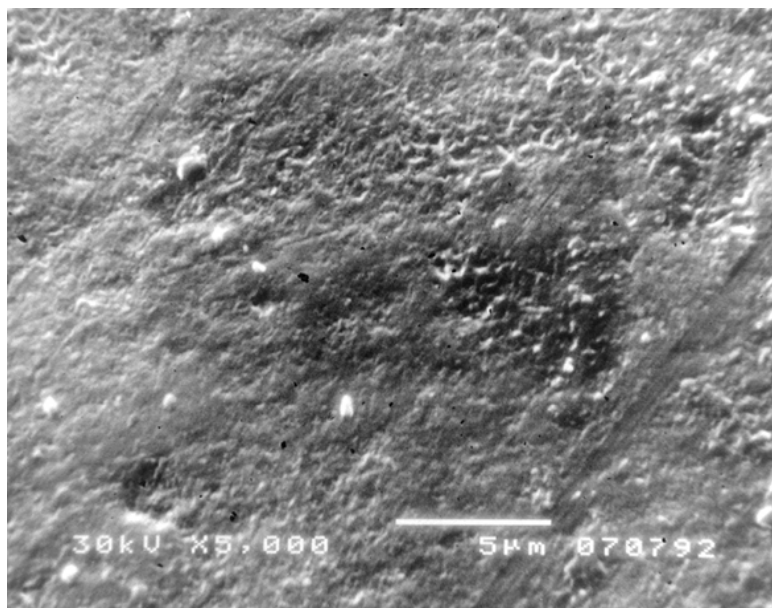


Figure. 3 Treated initial lesion of maxillary incisor

On Fig.3 can be seen the advanced, almost complete remineralization of the initial lesion (white spot), where the enamel residue is similar to the surface of the healthy enamel (magnifying 5000 times respectively).

♦ Ultrastructural changes of the untreated superficial lesion (maxillary primary incisors)

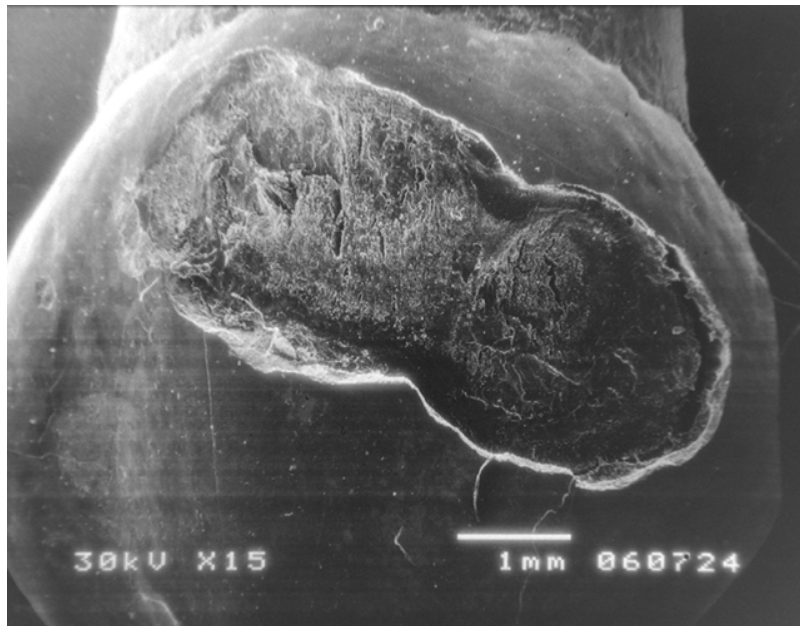


Figure. 4 Typical example of circulatory caries of maxillary incisor

On Fig.4 with a slight increase (750 times) of circulatory caries, limited by a healthy surrounding enamel.

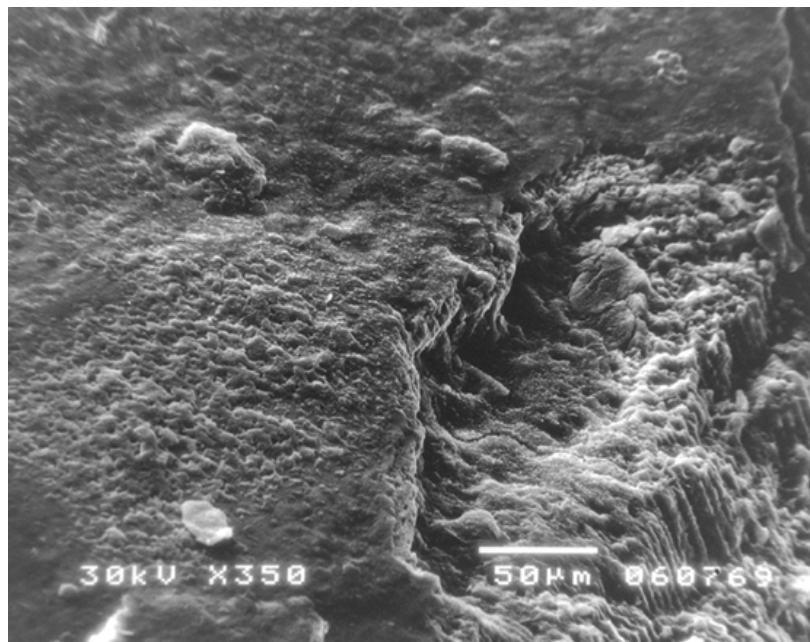


Figure. 5 Superficial lesion penetration at several levels

Figure 5 shows an interesting photo showing part of a healthy enamel cuticle and the penetration of a caries lesion on several levels with the loss of prismatic ridges (magnifying 350 times respectively).

3. Ultrastructural changes of treated teeth with topical fluoride treatment (maxillary incisors) with superficial lesion

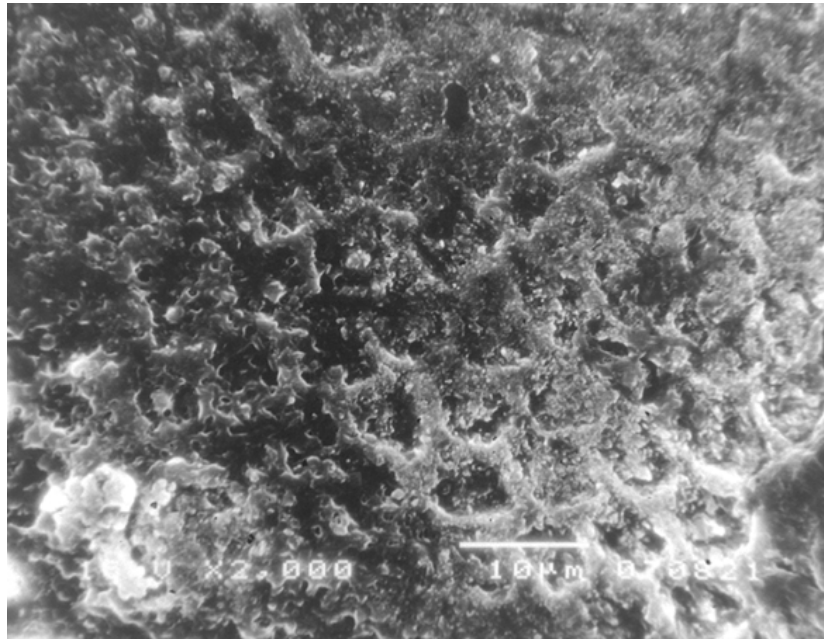


Figure. 6 Treated superficial lesion of maxillary incisor

In Fig. 6 the black fields on the left side are a complete destruction of the enamel prisms with wide inter-prismatic spaces, and on the right side is a filling, i.e. stopping of this destructive process. (magnifying 5000 times respectively).

Discussion

Enamel is made of hydroxyl apatite crystals arranged in prisms which extend from the dentin-enamel junction to the surface¹¹. Macroscopically crystals are packed tightly with inter crystalline spaces filled with water and organic material¹⁵. ECC appears very early in babies from 6 months, it progress rapidly, and often affects all four upper incisors. If the deposits accumulate over a longer period of time on the tooth surfaces, we do not have oral hygiene or the presence of fluorides, demineralization processes will dominate, which leads to cracking of the surface layer of the enamel and the initial lesion (white spot) at the level of the enamel, and then progresses in

width and depth through the dentin. So the chances of a white spot being demineralized,¹² or how long it will remain subclinical before programming into a clinically visible carious lesion, will depend on a number of factors, including the fact that caries is a multicellular⁹ disease. How successful remineralization will be, depends on the predominant, protective, or pathogenic factors^{14, 16}.

The question arises when it is best and most effective for the dentist to begin local fluoride therapy. Considering the fact that the enamel surface of the newly erupted enamel is not yet fully mineralized, and the teeth are most sensitive to cariogenic nicks in the first few months of eruption, local treatment should begin at age of two years, when most of the deciduous teeth are present and have already erupted¹⁷. There are various local fluoride agents in various forms: toothpastes, rinsing agents, solutions, gels, jellies, varnishes, etc. However, it is necessary to emphasize that the frequency of the preparation should be directly

related to the patient's risk of caries, and the choice of fluoride preparation should be the choice of the dentist. The application of the primary preventive measures can successfully prevent the Early Childhood Caries, and parents and the dentists, have the important role in that process, too.^{18,19} Avoiding food high in sugar, leaving baby bottle at 12-18 month of age, dental screening, counseling and preventive procedures are some of the measures and policies for EEC10,¹⁷. Best professional preventive method a dentist can use is local fluoride therapy²⁰, when tooth surface is periodically varnished with fluoride solution¹⁷. Best composition, concentrate of fluor and capacity for penetration in dental enamel are still investigate¹⁸. Fidy et al.²¹ studied the difference in penetration of Nano-NaF compared to ordinary NaF solution, and found that Nano-solution application can increase the levels of fluoride (0.1289%) and fluorapatite (20.35%) more than NaF application. Both fluoride solutions proved their influence for enamel endurance toward caries.

Diagnosing the disease in the early phase of clinical evolution – white spot lesion is very important, and the implementation of the primary preventive measures can achieve the biological reparation of the lesion and prevent the carious lesion extending and complications.^{2, 16,, 22, 23}. Unfortunately in most cases, the Early Childhood Caries findings are in the advanced phase, and the dentist usually can't manage the consequences².

Conclusions

From the overall SEM analysis of the evaluation of the ultrastructural changes of the enamel dental substance in untreated and treated dental specimens with local fluoride therapy, it is concluded:

- ♦ The appearance of ultrastructural changes in the enamel is correlated with the loss of minerals in the tooth substance
- ♦ The initial lesion is followed by demineralization and retraction of the prismatic ridges, destruction of the crystals and alteration of their arrangement in the prisms, expansion of the interprismatic spaces between the prisms and a multitude of dark spaces that are more pronounced in a deeper initial lesion.
- ♦ In the treated teeth with local fluoride treatment the interprismatic spaces are narrowed, the prismatic ridges are repaired and the surface of the enamel is leveled

We can also conclude that consumption of fluoride supplements, varnishes and fluoride tooth pastes must be provided. The application of the primary preventive measures can successfully prevent the Early Childhood Caries, and parents and the dentists, have the important role in that process. Best professional preventive method a dentist can use is local fluoride treatment, when tooth surface is periodically varnished with fluoride solution. All above can help in creation of the strategy for successful prevention of these disease.

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