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THE ROLE OF PROTECTIVE VARNISHES IN THE TREATMENT OF INITIAL CARIES

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Abstract: Objective: The aim of this study was to examine the impact of fluoride varnish and a new generation of nanoapatite varnish containing both Casein PhosphoPeptide − Amorphous Calcium Phosphate (CPP-ACP) and fluoride, on the activity of the initial smooth surface caries lesions of primary teeth.

Materials and Methods: A randomized clinical trial was conducted on a total of 120 primary teeth with initial caries lesions in enamel, at children under the age of 24 months. Clinical evaluation of initial carious lesions and its evaluation during the research was carried out using the International Caries Detection and Assessment System (ICDAS-II).

Results: There was a change of activity of the carious lesion in all three groups (CPP-ACPFG, FG and KG) during the time of treatment and follow-up, which was statistically significant. However, in the group of CPP-ACPFG, there was a better result compared to the FG and KG.

Conclusion: Remineralizing treatment of new generation varnish containing both nanoapatite and fluoride gave a better result compared to conventional varnish with fluoride.

Keywords: Fluor varnish, Casein PhosphoPeptide − Amorphous Calcium Phosphate, initial carious lesion, remineralization.

1. INTRODUCTION

The concept of minimally invasive therapeutic approach in dentistry, which is in the focus of the researches in recent years, aims to maximize the preservation of healthy tooth substance. Contemporary approach to prevention, the possibility of determining the risk prior to the occurrence of dental caries, new methods of diagnostics of disease in early stages (without caries cavity formed), have contributed to the modification of the therapeutic restorative treatment [1]. Modern resin based materials for the restoration of teeth, a better understanding of the pathogenesis of the disease, as well as the dynamics of the remineralization and demineralization process, accelerated the evolution of Black's principle „extension due to retention" to minimally invasive therapeutic approach [2].

The priority is to preserve a tooth structure primarily due to prevention, then, to stop further progression of the disease by minimal preparation and removal of the least possible amount of healthy tooth structure. By timely diagnosis of carious process at the level of „white spot" or „initial caries" lesion, it is possible to take proper prophylactic and therapeutic measures and lead to remineralization, to stop the further progression, thus avoiding the need for conventional restorative treatment [3]. Prevention and early treatment of carious lesions is extremely important, especially in early children's age, considering that children with advanced forms of the disease can experience great pain, eating and sleeping disorders, which can affect the overall health and development [4].

Nowadays, there are many ways to improve the protection of teeth. In addition to a toothbrush (classic, interdental, single, electric), dental floss, toothpaste, mouthwash, gel and special cream (GC Tooth Mousse), in recent decades dental varnishes have been used for teeth protection. Their use began in 1960 with the aim of providing a source of fluoride for caries prevention [5]. Many studies have confirmed the preventive fraction of dental varnish with fluoride, which is around 46% [6]. They have been widely used in Western Europe, Canada and the Scandinavian countries since 1980 as a tool of a prevention as well as a mean of remineralization therapy of initial caries lesions [7]. Recently, new technology has emerged based on the production of varnish

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which contains ions of calcium, phosphate and fluoride, in the form of nanoparticles [8].

The aim of this study was to investigate the effects of conventional varnish with fluoride and a new generation of nanoapatite containing Casein PhosphoPeptide − Amorphous Calcium Phosphate (CPP-ACP) and fluorine on the activity of the initial smooth surface caries lesions of primary teeth.

2. MATERIALS AND METHODS

2.1. Formation of the Sample

The study was conducted on the principle of randomized clinical trial on a total of 120 primary teeth with initial caries lesions in enamel, at children under the age of 24 months who attended regular pediatric controls at the Center of pediatric counseling at Public Health Centre, Banja Luka. Prior to start of the study, necessary approval of the Ethics Committee and the Director of Public Health Centre of Banja Luka was obtained. The survey was conducted between July 2012 and July 2013. Respondents were selected randomly. Healthy children who have been visiting regular pediatric checkups at the Centre of pediatric counseling, have been sent to the first dental examination by a specialist of pediatric and preventive dentistry.

The first dental examination of children was carried out in a separate office, within the Center of pediatric counseling, by one researcher, while each subsequent phase of the research was carried out at the Faculty of Medicine, Banja Luka. Children who were recommended by pediatricians were examined. Only those who passed the inclusion criteria were included in the further course of the investigation. The study included children, who, in addition to the possible use of children's toothpaste (local effect of about 500ppmF) were not using any other source of fluoride. Inclusion criteria were: healthy children without general and local diseases, teeth with active initial caries lesions without cavities formed, written parental consent for participation in research. Exclusion criteria were (absolute contraindication): children with allergies to colophony, dairy products and other types of allergy.

2.2. Study protocol

The selected tooth was completely wiped clean of plaque and dried by means of sterile gauze. Clinical evaluation of initial carious lesions at base line and their evaluation during the research was carried out using the International Caries Detection and Assessment System (ICDAS-II) (Table 1) [9,10]. Clinical progression of initial caries lesion (further demineralization) or its regression (remineralization), as well as its activity (active or inactive) was monitored during the period of time (Figure 1). Surface changes of enamel lesions in terms of roughness − active caries lesions or smooth surfaces - inactive caries lesion, was estimated by graduated periodontal probe (WHO, American Eagle).

In the study, we used two types of protective dental varnishes: conventional varnish containing fluoride, at a concentration of 1000 ppm F and contemporary varnish with Casein PhosphoPeptide − Amorphous Calcium Phosphate (CPP-ACP) and 5% sodium fluoride (22.6 ppm F in 1 ml).

Deciduous teeth that have undergone the inclusion criteria (120 teeth) were divided into three groups:

The first group or fluoride group − FG (38 teeth), was protected by fluoride varnish (1000 ppm F − 0.1%).

The second group or the Casein PhosphoPeptide − Amorphous Calcium Phosphate group − CPP-ACPF G (48 teeth) was protected by the varnish comprising a Casein PhosphoPeptide − Amorphous Calcium Phosphate and fluoride (5%, 22 600 ppm F at 1 ml).

The third group or the control group - KG (34 teeth), oral − health education of parents was conducted.

The study was conducted in four phases:

Phase I

Evaluation of activity of the initial carious lesion, parental training about proper oral hygiene in infants and the first varnish application were conducted.

Phase II

The second phase of the Research followed 10 days after the first phase. Parents and children were invited for remotivation and reapplication of varnishes (the second application).

Phase III

The third phase of the Research was 20 days after the first phase. Parents and children were invited for remotivation and reapplication of varnishes (third application).

Phase IV

The fourth phase was performed three months after the first phase when clinical evaluation of achieved results was performed.

Table 1. Clinical evaluation of initial caries lesions using ICDAS II system

ICDAS II	The clinical characteristics of the initial caries lesion	
	Active carious lesion	Inactive carious lesion
1	White or pale yellow opacity of enamel, dull, rough surfaces visible for 5 seconds after drying of enamel	White or pale yellow opacity of enamel, smooth and shiny, visible for 5 seconds after drying of enamel
2	Chalky white or yellowish lesions of enamel, opaque, rough, dull, visible in enamel without drying.	White or pale yellow (brownish) opacity, smooth and shiny, visible in the enamel without its drying
3	Opacity or color changes of enamel (chalky white, yellowish), opaque, rough, dull, visible without drying, and 5 seconds after drying is observed, undermined is the integrity of the enamel without visible dentin	Opacity or color changes of enamel (whitish, yellowish), smooth, shiny, visible without drying, and 5 seconds after drying is observed, undermined is the integrity of the enamel without visible dentin

*ICDAS 0 - healthy tooth

Figure 1. Active initial caries lesions in primary teeth by ICDAS II classification

2.3. Statistical analysis

For statistical analysis and presentation of results, used was SPSS 16.0 for Windows, MS Office Word and Excel MS Office. All results are presented numerically in tables and graphs. Qualitative characteristics are shown in the frequency and percentage representation. Quantitative characteristics are shown through basic indicators of descriptive statistics. In order to estimate population size, the resulting percentages and measures of central tendency, 95% confidence intervals were calculated. When it comes to statistics significance, connection between two categorical variables was tested by hi-square test.

3. RESULTS

The total number of teeth in the tested sample amounted to 120. Mostly, there were represented central incisors of the upper jaw, namely teeth 51 and 61 (19.2%), followed by the others (Table 2).

Results showed that in all three groups (CPP-ACPFG, FG and KG) during the time of treatment and follow-up, there was a change of lesion activity, which was statistically significant. However in the CPP-ACPFG group, there was a better result compared to FG and KG (Table 3).

Graphic can be represented as follows, where we can see that CPP-ACPFG has the most positive effect, followed by FG (Figure 2).

During the implementation of the research, parents of all three groups were trained in verbal and written form how to improve oral hygiene of tested teeth. Improvement was seen in all three groups using the Index of dental bio film. Reduction of the bio film was evident in all three groups as listed in Table 4. The decreasing trend was similar for all treatment groups - there was no difference among them.

Table 2. Description of the sample

Tooth	N	%
51	23	(19.2%)
61	23	(19.2%)
52	21	(17.5%)
62	19	(15.8%)
63	12	(10.0%)
53	10	(8.3%)
64	5	(4.2%)
54	4	(3.3%)
83	2	(1.7%)
73	1	(0.8%)

Table 3. Change of activity of initial caries lesions during the follow-up period

	CPP-ACPFG				FG				KG				
	Active		Inactive		Active		Inactive		Active		Inactive		
Phase	*N*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*N*	*%*	P2[2]
I	48	100,0	0	0,0	38	100,0	0	0,0	34	100,0	0	0,0	1,00
II	41	85,4	7	14,6	37	97,4	1	2,6	34	100,0	0	0,0	.007*
III	30	62,5	18	37,5	28	73,7	10	26,3	34	100,0	0	0,0	<.001*
IV	25	52,1	23	47,9	26	68,4	12	31,6	29	85,3	5	14,7	.016*
P1[1]	<.001*				<.001*				.004*				

1 Tested change over time within a single treatment group (Cochran's Q test). 2 tested the differences between treatments within a single visit

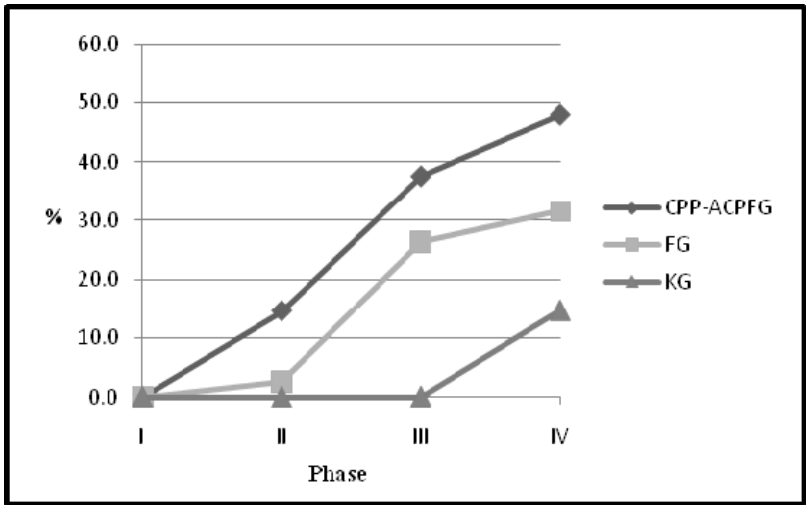

Figure 2. Change in activity of the treated lesions by the groups

Table 4. Values of the Index of dental bio film

Phase	Treatment			P[2]
	CPP−ACPFG	FG	KG	
I	1,90	2,00	2,29	,057
II	1,08	0,76	1,09	,093
III	0,58	0,32	0,53	,247
IV	0,56	0,42	0,76	,173
P[1]	<.001*	<.001*	<.001*	

[1] P value calculated based on the Friedman's test differences over time within each treatment groups. 2 P value calculated on the basis of the Kruskal-Wallis test of the differences between groups.

4. DISCUSSION

The investigation of initial caries lesion activity during the treatment and follow-up period showed that in all three groups (CPP-ACPFG, FG and KG) a degree of remineralization was achieved, which was statistically significant. However, in the group of CPP-ACPFG, there was a better result compared to the other two groups. In KG, improvement occurred somewhat later (only at the IV visit), suggesting that the implemented dental varnish treatment gave positive result in relation to the control group, where only oral – health education of parents was carried out.

During the implementation of the research, parents of all three groups were educated how to maintain oral hygiene at their offspring and how to remove dental bio film properly. The improvement was seen in all three groups by means of Index of the dental bio film. Reduction of bio film was visible in teeth of all three groups. This may be possible reason to some degree of improvement in lesion activity at control group, however, for better remineralization effect in addition to a consistent implementation of oral hygiene, it is necessary to apply appropriate protective agent to encourage recovery of demineralized enamel.

The most widely used and most studied, so far, in the research is varnish with fluoride concentrations of 22 600 ppm F, and many studies confirmed its preventive effect on dental caries [11−12]. It was also found that dental varnish with fluoride is far more effective than gels with amine fluoride (50% reduction for the varnish and 30% for the gel) [11−12]. Protective dental varnish is made for local and professional application. Although it may contain high concentrations of fluorine (22 600 ppm F), its usage is very safe, because a small amount (thin layer) is applied to individual teeth and hence it is released gradually. Researches have shown that the amount of fluorine in blood plasma after application of varnish onto the teeth in children aged two years old is just a seventh part comparing after application of gel with 1.2% fluoride [13−14].

Several studies have documented the occurrence of hypersensitivity reactions (contact dermatitis) in patients who have an allergy to resin [15−17]. Neither study did confirm the acute toxic effects of professional use of fluoride varnish in children [18].

The main preventive effect of dental varnish is to encourage remineralization of initial caries lesions. It also has the advantage over other forms of topical fluoridation because they are more physically present on the tooth, and hence facilitate the conversion of calcium fluoride in the fluorine apatite. Fluorine originating from varnishes is proven to encourage redistribution of ions in the crystal lattice, reducing porosity and size of lesion in enamel [18].

There are many studies showing correlation between the reduction of caries in the primary dentition and the use of fluoride varnish [19−22]. Weintraub and colleagues at study conducted in the United States for a period of two years engaged in protection of teeth in high-risk (Hispanic and Chinese origin) preschool children, achieved reduction of dental caries by 62.5% [6].

Lawrence et al. have reached the reduction of dental caries about 24% in high-risk populations aged six months to five years old [20].

Marinho and colleagues, at total of 2 709 children and adolescents treated with dental varnish, found that preventive fraction of caries lesion was 46% for the permanent dentition, and for primary 33%, compared to the control group [5].

Carvalho et al. have reviewed the available electronic databases (BBO, LILACS, Medline, Cochrane) and selected 513 scientific articles where they found that preventive fraction varied from 5% to 63% [21].

Demineralization of enamel is simply defined as loss of calcium and phosphate and consequently creation of initial carious lesions. This initial lesion is reversible and disappears within a process that is opposed to demineralization which is called remineralization − diffusion of calcium and phosphate ions back into the enamel [18]. It has been proved that some dairy products have the ability of compensation of these ions. Based on this, Professor Reynolds in his *in situ* studies, demonstrated that exposure of enamel to peptides from milk protein – casein, led to a significant reduction of demineralization [23−26].

Casein PhosphoPeptide − Amorphous Calcium Phosphate was first mentioned in 1998, after that followed numerous studies on animals and on humans (mainly *in situ*) proving its anti − cariogenic activity [23]. Since then, it has been used in food and dental products for cavity prevention and promotion of tooth remineralization.

It was found that chewing gum with CPP-ACP shows twice the remineralizing properties than ordinary chewing sugar-free gum [27].

Walker and associates in their experiments showed the influence of consumption of cow's milk with or without added CPP-ACP on the remineralization of lesions in enamel. Ten respondents daily consumed 200 ml of milk contained 2.0−5.0 g CPP-ACP / l, while the control group consumed milk without this additive. After three weeks, a statistically significant difference was demonstrated

in the remineralization of initial lesions in subjects who drank milk with CPP-ACP [28].

Incorporation of Casein PhosphoPeptide − Amorphous Calcium Phosphate into the tooth filling material, Glass ionomer cement (GCJ), demonstrated a significant increase in release of ions as calcium, phosphate and fluoride into the tooth structure, which increased resistance toward acid effects [29].

More recent research shows efficient installation of minerals in the dental bio film, and thus in the enamel, if the product for oral use contains both of 2% CPP-ACP and fluorine at a concentration of at least 1100 ppm. This provides a better remineralizing properties compared to the individual effect of CPP-ACP or F [26]. Based on this, a new generation varnish has been produced, containing 5% sodium fluoride and Casein Phospho Peptide − Amorphous Calcium Phosphate. In this way, protective effect of fluoride is enhanced, and in the presence of calcium and phosphate, resistance of enamel against demineralization and caries is strengthened [30].

This is a relatively new technology and there is still no available researches, especially at primary dentition. In this study, for the first time in therapy of white spot lesions in early caries at deciduous teeth *in vivo*, in addition to conventional varnish with fluoride, a new generation Casein PhosphoPeptide - Amorphous Calcium Phosphate and fluoride varnish was investigated.

5. CONCLUSION

Considering the limitations of this study, we can conclude that remineralizing treatment with the new generation nanoapatite and fluorine containing varnish shows a better result compared to conventional varnish with fluoride. However, a certain improvement of clinical symptoms occurred in the case of the control group, owing to the consistent removal of dental bio film. Further research is required to clarify remineralization effects of varnishes on the primary teeth enamel.

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УЛОГА ПРОТЕКТИВНИХ ЛАКОВА У ТЕРАПИЈИ ПОЧЕТНОГ КАРИЈЕСА

Сажетак: Циљ: циљ истраживања је био испитати утицај лака са флуором и лака нове генерације који садржи наноапатит казеин фосфопептид − аморфни калцијум-фосфат (CPP-ACP) и флуор на активност почетне кариозне лезије глатких површина млијечних зуба.

Материјал и методе: рандомизирано клиничко истраживање спроведено је на укупно 120 млијечних зуба са почетним кариозним лезијама у глеђи, дјеце узраста до 24 мјесеца. Клиничка процјена почетне кариозне лезије и њена евалуација током истраживања вршена је помоћу International Caries Detection and Assessment System (ICDAS-II).

Резултати: испитивањем активности почетне кариозне лезије дошло се до резултата да је у све три испитиване групе (CPP-ACPFG, FG и KG) током времена третмана и праћења дошло до промјене активности лезије, што је статистички значајно. Међутим, у групи CPP-ACPFG постоји бољи резултат у односу на FG и KG.

Закључак: Реминерализирајући третман лаковима нове генерације који садрже наноапатит и флуор дао је бољи резултат у односу на конвенционални лак са флуором.

Кључне ријечи: лак са флуором, казеин фосфопептид − аморфни калцијум--фосфат, почетне кариозне лезије, реминерализација.