

Chlorhexidine과 fluoride 성분의 varnish가 타액 내 mutans streptococci 수에 미치는 효과

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국문초록

본 연구의 목적은 chlorhexidine(Cervitec)과 fluoride(Fluor-protector) 성분을 각각 포함한 varnish를 치아에 적용하였을 때 타액내의 mutans streptococci의 수의 감소에 미치는 효과를 알아보기 위한 것이다.

전북대학교 치과대학생 40명을 대상으로 하여 mutans streptococci 균종 수를 평가하였으며, 이들을 무작위로 네 군으로 나누어 다음과 같이 varnish를 적용하였다: A) varnish를 적용하지 않은 군(n=10), B) chlorhexidine varnish 적용군(n=10), C) fluoride varnish 적용군(n=10), D) chlorhexidine과 fluoride varnish를 모두 적용한 군(n=10). Mutans streptococci 수 측정에는 Orion diagnostica 사의 Dentocult SM을 사용하였다. Varnish를 적용하지 않은 상태에서 1차로 mutans streptococci 수를 측정한 후 각각의 varnish를 적용하였으며, varnish 적용 12주 후 다시 측정하여 기록하였다.

모든 실험군(group B, C, D)에서 12주 후에 측정한 mutans streptococci 수가 감소한 개체가 증가한 개체보다 많았으나 통계적으로 유의한 차이는 보이지 않았다($p>0.05$). 실험에 사용된 어떠한 varnish도 적용 12주 후에 타액 내 mutans streptococci 수에 통계적으로 유의할 만한 변화를 주지는 못하는 것으로 나타났다($p>0.05$).

주요어 : 클로르헥시딘, 불소 바니쉬, 뮤탄스 연쇄상구균

I. Introduction

The prevalence of dental caries has gradually decreased in past years, but caries prevalence is still very high^{1,2)} in industrial country. Thus, prevention of dental caries is still an important challenge in modern oral health science.

Mutans streptococci(MS) are considered as a major caries-risk factor³⁾ in the development both of initial caries lesions and recurrent decay⁴⁾.

Chlorhexidine has been widely accepted as an

efficient tool in the prevention of dental caries. Chlorhexidine varnish have been reported to reduce the number of MS in plaque and saliva for considerable periods of time, and this long-lasting effect is probably due to the prolonged contact time between varnish and teeth⁵⁻⁸⁾.

Petersson et al.⁹⁾ and Twetman and Petersson¹⁰⁾ reported a significant suppression of mutans streptococci in plaque samples collected from interproximal sites over a 3-month-period following two successive applications of Cervitec. Attin et al.¹¹⁾ reported Cervitec did not kill mutans streptococci effectively. Kristoffersson and Bratthall¹²⁾ also evaluated the effect of 1% chlorhexidine gel on mutans streptococci, reported mutans streptococci returned back to pre-treatment levels at most approximal sites after 40 days.

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Fluoride plays an important role in dental caries prevention, mainly due to its effect on the calcified tissues of teeth. However, an additional important preventive effect of fluoride is its ability to reduce acid formation in some bacterial species of dental plaque e.g. mutans streptococci. It has been shown that for mutans streptococci under glucose excess and at low environmental pH, the accumulation of biofilm cells on fluoride-bound hydroxyapatite was significantly reduced¹³. Topical treatment with fluoride varnish have been proposed what reduce and control approximal caries development.

It is generally thought that fluoride has an additive effect on chlorhexidine treatments¹⁴ and a prolonged suppression of salivary mutans streptococci levels has been reported after chlorhexidine and fluoride gel treatments¹⁵. Furthermore, a caries-inhibiting effect of a combined chlorhexidine and NaF rinse has been suggested¹⁶. A combination of an antibacterial and fluoride varnish could therefore be of clinical interest. Such a mixture has recently been investigated in artificial demineralization systems with encouraging results^{17,18}.

The aim of this study was to compare the efficacy of the chlorhexidine(Cervitec), fluoride(Fluor-protector) and combination of chlorhexidine and fluoride varnishes in decreasing the level of salivary mutans streptococci.

II. Materials and Methods

1. Subjects

40 students of school of dentistry, Chonbuk national university were selected for this study. Their mean age was 26.1-years-old. They were randomized into four different groups as what varnishes they were treated with: (1) untreated group (n=10), (2) chlorhexidine varnish group(Ch) (n=10), (3) fluoride group(F) (n=10), (4) combination of chlorhexidine and fluoride varnish group(ChF) (n=10): identified with the chair-side strip mutans method, were analysed separately.

2. Oral examination and salivary analysis

Caries experience of each subject were evaluated

with DMFS index according to WHO criteria. At the same time, analysis of the baseline salivary mutans streptococci counts(MS1) was also performed after the oral examination. No eating, drinking or smoking was allowed for 1 hour before the treatment. The salivary MS score were determined with the saliva strip mutans technique according to Jensen and Brattall¹⁹ with commercially available strip test kit(Dentocult SM, Orion Diagnostica, Finland). Stimulated saliva with paraffin wax for a minute were used for test. The strips were cultivated for 48 hours in 37°C and evaluated and scored with naked eye. The number of colony-forming units(CFU) with characteristic morphology was screened and scored 0-3. Subjects with score 0 or 1 corresponds to $<10^5$ CFU/ml saliva. Score 2 and score 3 corresponds to 10^5 - 10^6 CFU/ml saliva and to $>10^6$ CFU/ml saliva.

3. Treatment

Prior to each varnish application, teeth were professionally cleaned with rubber cup and pumice paste. The interdental areas were cleaned with unwaxed dental floss. Each quadrant was isolated with cotton rolls and dried with compressed air. Chlorhexidine varnish group was treated with Cervitec(1% chlorhexidine and 1% thymol, Vivadent, Schaan, Liechtenstein), and a thin coat of varnish was applied to all present teeth with a brush, especially interproximal areas with unwaxed floss. Cotton rolls were removed after 30 seconds. The chlorhexidine group were instructed not to eat or drink for 3 hours and not to brush their teeth for 24 hours and not to floss or use toothpicks in all treated area for 3 days. Fluoride varnish group was treated with Fluor-protector(0.1% fluoride, Ivoclar-Vivadent), which was applied to all present teeth in accordance with the manufacturer's instructions for 60 seconds. Eating, drinking and toothbrushing were forbidden for 45 minutes. In the combination group, chlorhexidine varnish was applied first and the treated teeth was overlaid with fluoride varnish with the methods in the manner described above, 1 week after the treatment with chlorhexidine varnish. Untreated group did not treated with any experimental varnishes.

12 weeks after the final varnish treatment in each group mutans streptococci counts in saliva were

recorded as described above. During the entire program, a single dentist carried out all the examinations, which were performed in a blinded fashion.

4. Statistical analysis

To compare the mutans streptococci counts Wilcoxon test was used to analyse. All microbiological measurements are observed on a grading scale, standard statistical methods like the t-test or the analysis of variance cannot be applied.

III. Results

Mean DMFS of all subjects was 12.88(SD=8.99). Mean baseline mutans streptococci counts(MS1) was 1.23(SD=0.92). Mean mutans streptococci counts evaluated 12 weeks after treatment with varnish

(MS2) was 0.98(SD=0.70)(Table 1). All experimental varnish groups-chlorhexidine, fluoride, combination of chlorhexidine and fluoride group-had decreased MS2 compared to MS1, but no significant differences were examined(p>0.05). There was no statistical difference between the three treatment groups(p>0.05).

Fig. 1, 2, 3 and 4 shows the MS1 and MS2 counts of each varnish group. Salivary mutans streptococci counts suppressed in 50% of the Chlorhexidine varnish group, especially the subjects with high MS1 level(MS2≥2) in 80%. But those varnishes tested had no statistically significant effect on salivary mutans streptococci value(p>0.05). Consequently the subjects with decreased mutans streptococci counts were more numerous than the subjects with increased mutans streptococci counts in the experimental groups. High level MS1 group(MS1≥2) were

Table 1. Mean MS1 and MS2 of the each varnish group

		Mean	S.D.	Mean MS2-MS1	Wilcoxon Difference			Sig.	N
					MS2<MS1	MS2>MS1	MS1=MS2		
un-treated	DMFS	12.40	8.40						
	MS1	0.90	0.99	0.20	2	5	3	0.527	10
	MS2	1.10	0.74						
Ch	DMFS	14.30	8.69						
	MS1	1.30	0.82	-0.40	5	2	3	0.206	10
	MS2	0.90	0.57						
F	DMFS	9.20	8.61						
	MS1	1.40	0.84	-0.40	3	2	5	0.336	10
	MS2	1.00	0.82						
ChF	DMFS	15.60	10.20						
	MS1	1.30	1.06	-0.40	3	1	6	0.194	10
	MS2	0.90	0.74						

Table 2. MS1 and MS2 in the subjects with high baseline MS score (MS1 score ≥ 2)

		Mean	S.D.	Mean MS2-MS1	Wilcoxon Difference			Sig.	N
					MS2<MS1	MS2>MS1	MS1=MS2		
Ch	DMFS	15.00	11.29						
	MS1	2.00	0.00	-1.00	4	0	1	0.059	5
	MS2	1.00	0.71						
F	DMFS	10.00	11.20						
	MS1	2.25	0.50	-1.25	2	0	2	0.18	4
	MS2	1.00	1.15						
ChF	DMFS	23.00	4.64						
	MS1	2.20	0.45	-1.00	3	0	2	0.102	5
	MS2	1.20	0.84						

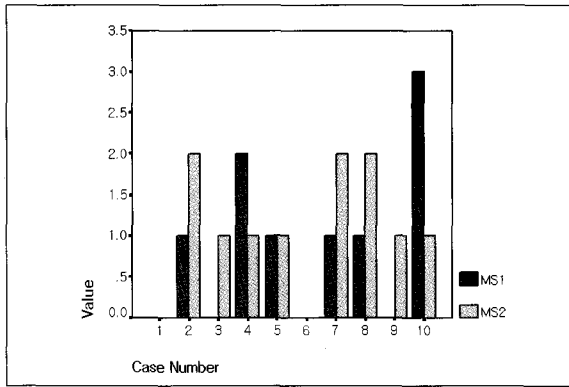


Fig. 1. placebo group.

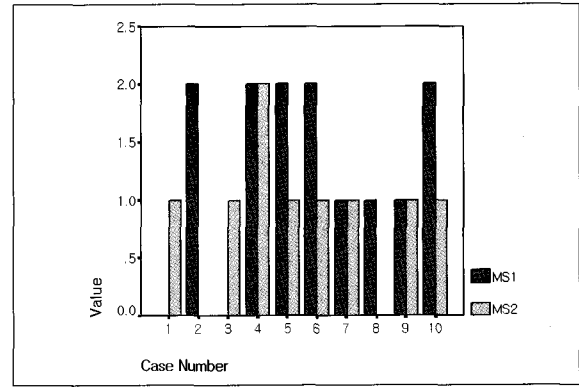


Fig. 2. Chlorhexidine varnish group.

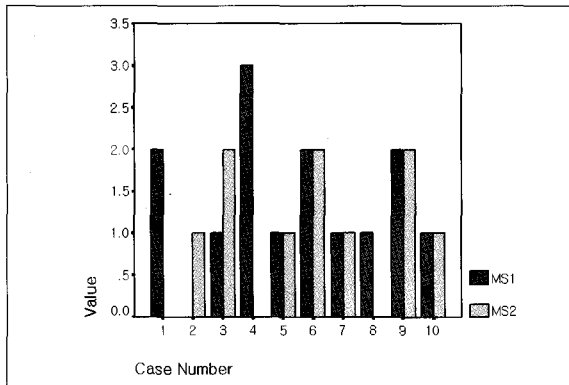


Fig. 3. Fluoride varnish group.

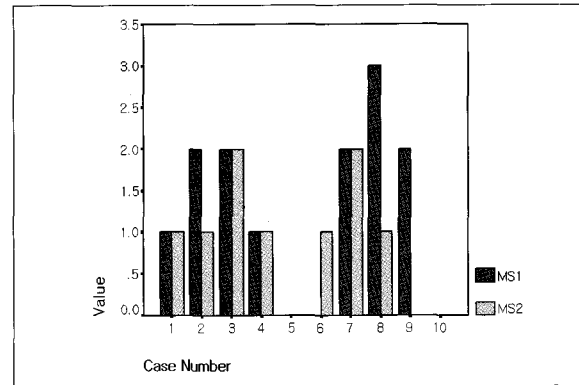


Fig. 4. Combination varnish group.

35%(14 subjects; untreated group: 2, chlorhexidine group: 5, fluoride group: 4, combination group: 5) of all investigated subjects.

The subjects with high baseline mutans streptococci score had more remarkable reduction in the level of mutans streptococci 12 weeks later(Table 2). But this result was not statistically significant($p>0.05$).

IV. Discussion

The present study was investigated to evaluate and compare the efficacy of the chlorhexidine (Cervitec), Fluoride(Fluor-protector) and combination of chlorhexidine and fluoride varnishes in decreasing the level of mutans streptococci 12 weeks after varnish applied.

There were a large number of previous studies about effects of chlorhexidine varnishes. Chlorhexidine varnish has been reported that is effective to reduce salivary and interdental mutans streptococci⁹⁾.

Short term-use of chlorhexidine at low concentrations has been reported to reduce temporarily the total microbial counts in plaque, but not to have any effect on total salivary counts²⁰⁾.

In present study, chlorhexidine varnish application did suppress salivary mutans streptococci counts in half of the chlorhexidine varnish group. Especially the subjects with high MS2 level had more decreased mutans streptococci counts. But those varnishes tested had no statistically significant effect on salivary mutans streptococci value.

Previous studies showed similar findings with lowly concentrated varnishes(Cervitec). Petersson et al.⁹⁾, reported that they found a gradual return to pre-treatment values at their 1- and 3-month examination. When Attin et al.¹¹⁾ examined interproximal plaque, chlorhexidine varnishes of high concentration(EC40, 40% chlorhexidine) lead to a reduction of high MS values in interdental plaque over a period of 12 weeks. This controversial finding can be explained

by the different plaque sampling locations, or the frequency of varnish application. Highly concentrated varnish a chlorhexidine reservoir might be formed in cracks and enamel porosities which is maintained even if the varnish is removed from the tooth surfaces after a contact time of 8 minutes. But they did not obtain any statistically significant result on effects of the low concentrated chlorhexidine varnish (Cervitec) on the salivary level of mutans streptococci after 12 weeks. Differently interproximal level of mutans streptococci were decreased significantly. The influence of the tongue and the saliva amount and composition in the lower jaw also could be an explanation for the reduced effect of Cervitec on mutans streptococci.

Fluoride has been suggested to act additively, or even synergistically, with chlorhexidine on mutans streptococci^{21,22)}. This study was planned, therefore, to evaluate any possible additional effects of a fluoride varnish on the reduction of salivary mutans streptococci. Balzar et al.²³⁾ reported that pretreatment of hydroxyapatite discs with fluoride varnish of fluoride solutions caused a statistically significant reduction of lactic acid formation in associated, growing biofilms of *streptococcus mutans*. In the present study, however, fluoride varnish had no statistically significant effect on salivary mutans streptococci.

Ostela et al.²⁴⁾ and Pienihakkinen et al.²⁵⁾ reported that when they measured mutans streptococci level by the adhesion-based strip method, recolonization appeared slower than when measured by the conventional laboratory analyses for MS and may be due to the inhibitory effect of chlorhexidine and fluoride on the adherence of MS. Other study¹⁰⁾ reported that mixture of a chlorhexidine and fluoride varnish was as effective as the chlorhexidine varnish in reducing interdental levels of mutans streptococci in the short perspective(1 month) and even slightly more effective after 3 months.

Other studies^{11,26,27)} treated the dentition several times with varnish for more effect. But in this study, the dentition was treated only once with the varnishes. Pienihakkinen et al.²⁵⁾ reported they treated the dentition only once with the varnish. Even with that procedure the varnish gave an equal or a longer suppression of salivary mutans streptococci than three treatments with the gel. Sandham et al.²⁸⁾ when test-

ing the application of chlorhexidine varnish on all tooth surfaces to suppress MS in saliva, observed that the effect was more successful in patients with a low DMFT score compared with those with a high score.

The reduction in the amount of plaque or any caries-preventive effects were not analyzed in the present study. Haukali et al.²⁷⁾ reported that their study did not indicate an effect of Cervitec on initiation or progression of approximal caries in a population with a low DMFS. Baca et al.²⁹⁾ reported similar results that the cessation for 3 years of a 3-month program of chlorhexidine-thymol varnish applications resulted in a non-significant increase in the prevalence of dental caries in the permanent first molar. Hicks et al.³⁰⁾ evaluated the effects of a fluoride-releasing resin designed for amalgam bonding on secondary caries formation in primary teeth restored with amalgams. They reported that the caries susceptibility of enamel surfaces adjacent to amalgams with the fluoride-releasing bonding agent was decreased considerably. Fontana et al.³¹⁾ studied the efficacy of fluoride varnish in inhibiting progression of secondary caries. their results suggest that the application of a fluoride varnish to early, active secondary carious lesions significantly slows down their progression. Furthermore they reported that the application of a placebo varnish showed a trend towards slowing down lesion progression, suggesting that the therapeutic effect of the varnish on secondary caries is not only due to its fluoride release. Eileen et al.³²⁾ reported that the fluoride releasing glass-ionomer restoration material demonstrated a statistically significant degree of protection of enamel from demineralization compared to the non-fluoride control material.

In conclusion, the chlorhexidine and fluoride containing varnishes that used in this study did not have any statistically significant effects on the level of mutans streptococci in saliva. However, caution must be taken in generalizing the results to other populations because of the small sample sizes, and the relatively good oral hygiene. Dental caries is a multi-factorial disease and there are lots of factors that would affect to the level of mutans streptococci. Thus there might be various results by the conditions during studies. More sample size and thorough oral

hygiene instruction might make the results of study more reliable. Even though there was no significant result, our findings warrant the implementation of further clinical studies to elucidate the topic.

V. Conclusion

We evaluated forty healthy students of school of dentistry, Chonbuk national university with strip method to compare the efficacy of a chlorhexidine, a fluoride and a combination of chlorhexidine and fluoride varnishes in decreasing the level of salivary mutans streptococci. Twelve weeks later the completion of each varnish treatment, we came to this conclusion.

In varnish group with chlorhexidine, fluoride and combination of chlorhexidine and fluoride, the level of mutans streptococci was lower after 12 weeks than at baseline, but there were no significant differences in saliva ($p > 0.05$), when compared with baseline. After 12 weeks, a remarkable reduction was still found in the subjects with high level of mutans streptococci at baseline, but not different in the low and moderate level of mutans streptococci ($p > 0.05$).

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Abstract

EFFECT OF THE CHLORHEXIDINE AND FLUORIDE-CONTAINING VARNISH
ON THE LEVEL OF SALIVARY MUTANS STREPTOCOCCI

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The objective of the present prospective trial was to compare the efficacy of chlorhexidine(Cervitec), Fluoride(Fluoroprotector) and combination of chlorhexidine and fluoride varnishes in decreasing the level of salivary mutans streptococci.

Forty healthy students of school of dentistry, Chonbuk national university were investigated to evaluate mutans streptococci(MS) counts and randomized into four groups to treat with the experimental varnishes: A) untreated group(n=10), B) chlorhexidine group(n=10), C) fluoride group(n=10), D) chlorhexidine and fluoride group(n=10). Dentocult SM[®](Orion Diagnostica) strip method was used for measurement of the level of mutans streptococci in saliva. Stimulated saliva were collected at baseline for mutans streptococci counts evaluation(ms1), 12 weeks later the completion of each varnish treatment, mutans streptococci counts were re-evaluated.

In varnish group with chlorhexidine, fluoride and combination of chlorhexidine and fluoride, the level of mutans streptococci was lower after 12 weeks than at baseline, but there were no significant differences in saliva($p>0.05$), when compared with baseline. After 12 weeks, a remarkable reduction was still found in the subjects with high level of mutans streptococci at baseline, but not different in the low and moderate level of mutans streptococci($p>0.05$).

Key words : Chlorhexidine, Fluoride varnishes, Mutans streptococci