

# The Effect of Calcium Pre-Rinse on Salivary Fluoride After 900 ppm Fluoride Mouthwash: A Randomized Clinical Trial

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## Abstract

**Objective:** Calcium fluoride deposit during fluoride application. Uptake and retention of fluoride by saliva depends generally on the concentration of calcium. In this study, the effect of calcium pre-rinse on salivary fluoride concentration after a 900 ppm fluoride mouthwash was investigated.

**Materials and Methods:** This cross-over double-blind randomized clinical trial was conducted in a girls' dormitory in Zahedan University of Medical Sciences, southeast Iran. In this study, 42 female dental students were chosen using simple randomization. During the first phase, 21 subjects (group A) used fluoride rinse (F regimen) and the remaining (group B) used calcium pre-rinse followed immediately by fluoride rinse (Ca + F regimen). In the second phase, participants rinsed using the mouthwashes not previously used. Prior to each phase prophylaxis was performed and no fluoridated product was used during a two-week interval between the phases. Salivary samples were taken immediately before (baseline), 1 and 12 hours after rinsing. The salivary fluoride concentration was determined using fluoride sensitive electrode. Repeated measures ANOVA was used for statistical analysis and the significance level was set at  $P < 0.05$ .

**Results:** There was significant difference between fluoride concentrations at different time points ( $P < 0.001$ ). Significant differences were observed when the different time points of two regimens were examined. In contrast to this, the baseline before using F regimen and the baseline before using Ca + F regimen did not show any significance ( $P = 0.070$ ).

**Conclusion:** Pre-rinsing with calcium before fluoride is recommended because of significant increases in salivary fluoride concentration.

**Key Words:** Calcium lactate; Fluorides; Mouthwashes; Saliva; Sodium Fluoride

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Received: 12 March 2013

Accepted: 24 May 2013

*Journal of Dentistry, Tehran University of Medical Sciences, Tehran, Iran (2013; Vol. 10, No.4)*

## INTRODUCTION

Frequent exposure to topical fluoride is of great importance in preventing or stopping dental caries. Moreover, in adjunct to the daily application of fluoride, optimal oral hygiene and concise control of diet are highly recom-

mended to optimize the effects of fluoride [1]. Benefits of fluoride include promoting the resistance of enamel to acid solubility, enhancing the process of remineralization and also the ability to weaken the cariogenic potential of microorganisms [2].

Since saliva acts as a vehicle for the delivery of fluoride to the dental plaque, the above mentioned mechanisms are highly dependent on salivary fluoride concentration [2, 3].

Despite a high increase in salivary and plaque fluoride concentration after using fluoridated agents, within 1 or 2 hours, the fluoride concentration decreases sharply compared to the baseline [2]. Therefore, it has been emphasized that fluoridated products such as mouthwashes should be used regularly.

During the topical use of fluoride, fluoride ion can result in  $\text{CaF}_2$  deposition on the tooth surface [4]. Some studies have shown that  $\text{CaF}_2$  accumulates not only on the tooth surface, but also to some extent it can be incorporated in the enamel structure [5]. The availability of calcium ion, which can be delivered from the enamel, saliva, and dental plaque fluid, is a critical factor for  $\text{CaF}_2$  formation [4]. The fluoride level of plaque increases after using fluoridated products. Due to the direct relationship between the fluoride and calcium concentration of the plaque, the ability of the plaque to maintain the fluoride ion can be extended by increasing the calcium concentration of the plaque. Unfortunately, during fluoride usage, the formation of  $\text{CaF}_2$  deposit is limited due to the low level of bio-available oral calcium in relation to the amount of delivered fluoride [6].  $\text{CaF}_2$  acts as a long term (two week) reservoir of fluoride ion on the tooth surface and plaque, and is considered as an important factor for the anti-caries potential of fluoride [7]. Several agents such as calcium chloride, calcium lactate, calcium phosphate, calcium glycerophosphate, and  $\alpha$ -tri calcium phosphate have been introduced in forms of chewing gum, dentifrice, and mouthwash at different concentrations [8].

In a study conducted by Whitford et al., the  $\text{CaCl}_2$  solution given before fluoride dentifrice had only minor effects on the salivary fluoride [9]. A study by Pessan et al. demonstrated that a calcium pre-rinse significantly increased the fluoride concentration after 1 hour of fluoride

dentifrice usage and this effect does not persist overnight [8]. Vogel et al. found that calcium pre-rinse used immediately before rinsing 228 ppm fluoride mouthwash had a significant impact on the 1-hour salivary fluoride (6-9 folded increase) [6]. In a recent study, Vogel et al. have presented data suggesting that calcium pre-treatment elevates salivary fluoride overnight and hence induces the cariostatic effect [10]. Studies have been investigating the effect of calcium rinse on the salivary fluoride concentration following 228 ppm fluoride mouth rinse (0.05% NaF) [6, 10]. The simultaneous use of calcium pre-rinse and 900 ppm fluoride rinse (0.2% NaF), which is usually used once a week, has yet to be investigated. The aim of this study was to evaluate the effect of calcium pre-rinse on salivary fluoride after 900 ppm fluoride mouthwash.

## MATERIALS AND METHODS

This study was approved by the Research and Ethics Committee of Zahedan University of Medical Sciences and it has been registered in the Iranian Registry of Clinical Trials (IRCT201107306105N2). Informed consent was given by the participants.

In this double-blind cross-over randomized clinical trial, inclusion criteria were being 18-24 years old, having good oral hygiene (Simplified Oral Hygiene Index  $\leq 1.2$ ) and a normal oral architecture (no clinical sign of inflammation, discoloration or any pathosis in the oral soft tissue observed in clinical examination), normal oral physiology and salivary gland function. Exclusion criteria included having untreated caries, enamel defects, systemic diseases, prosthesis, orthodontic appliances, and consumption of drugs that affect the quality or quantity of saliva and history of consuming antibiotics or antibacterial mouthwashes during the 2 weeks prior to the examination. After preparing a list of all female dental students who lived in the university dormitory and had the eligibility criteria, 42 students were randomly chosen from 98 students using

the table of random numbers ( $z_{1-\alpha/2}=1.96$ ,  $z_{1-\beta}=0.85$ ,  $d=1.3$ ,  $s_d=3$ ) and were randomly divided into A and B groups. The study was performed in two phases.

#### First phase:

After dental prophylaxis, participants ( $n=42$ ) were asked not to use fluoridated dentifrice, mouth wash, dental floss and any other form of fluoride during the next two weeks. Only tooth brush and floss with unfluoridated products were allowed. During the last 24 hours before using the mouthwash, the participants were instructed to avoid any food or drink containing fluoride such as tea.

The first saliva samples (baseline) were taken in the dormitory at 7-8 pm, and at least 1 hour after food ingestion.

For collecting the saliva samples, the participants were asked to sit in an upright and resting position, and expectorated the unstimulated saliva into a plastic vial for 3 minutes. Twenty one subjects (group A) were then given 10ml of 900 ppm fluoride mouthwash (0.2% NaF, Behsa, Iran) and were then instructed to rinse for 1 minute (F regimen). Whilst the remaining subjects (group B) were asked to rinse with 10ml of calcium mouthwash (160 mmol/l calcium lactate pentahydrate, Merk, Germany) for 1 minute and then immediately rinse with 10ml of 900 ppm fluoride rinse (0.2% NaF, Behsa, Iran) for the same duration (Ca + F regimen). One hour (at 8-9 pm), and 12 hours after using mouthrinses (the next morning at 7-8 am), unstimulated saliva was collected once again. No food, beverage (except water), chewing gum, or oral hygiene measures were allowed during this 12-hour period.

#### Second phase:

This phase lasted two weeks after the end of the first phase. This period was considered because gradual loss of  $\text{CaF}_2$  occurs after two weeks [11]. Just like the first phase, after dental prophylaxis, all required recommendations about the two weeks and one day prior to rins-

ing, were repeated. After two weeks, the participants ( $n=42$ ) were given the rinsing not previously used in the first phase. Saliva collection was performed like the first phase at the same time as baseline, 1, and 12 hours after rinsing.

In this study, a dental student who lived in this dormitory assessed the other students for eligibility, enrolled them, chose the participants and divided them randomly into two groups and supervised them, registered the time, administered mouthwashes, and did the sampling at exact times. It should also be mentioned that in both phases, the participants had the same dinner at the same time. Recruitment was done in February 2011 for a one-month period and all the volunteers remained throughout the study. Collected samples were preserved at a temperature of  $-20^\circ\text{C}$ . The technique described by Vogel et al. [10] was applied to prepare the saliva samples. The diluted samples and standard fluoride solutions were analyzed using the ion fluoride selective electrode (Metrohm Co, Swiss) calibrated with standard samples. The analyzer and participants were blind to the type of mouthwash.

#### Statistical Analysis

SPSS 18 for Windows (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. Repeated measures ANOVA was used for statistical analysis of salivary fluoride concentration and  $P < 0.05$  was considered significant. We considered the time of sampling as one factor and the mouth rinse regimen as another factor for data analysis.

#### RESULTS

The mean age ( $\pm\text{SD}$ ) of the participants was  $19.79 \pm 1.44$  years. The average flow rate of saliva at different times ranged from 1.41 to 1.85 ml/min and did not differ significantly. The fluoride concentration 1 hour after using both rinses (Ca + F regimen) was the highest observed.

Table 1 shows the average salivary fluoride concentration at baseline, 1, and 12 hours after rinsing. According to repeated measures ANOVA, there was a significant difference between the concentrations of fluoride measured at different time points ( $P < 0.001$ ). Fluoride concentration increased by 6.25 and 7.42 times in 1 and 12 hours, respectively after rinsing with calcium and fluoride mouthwashes. Pairwise comparison of fluoride concentrations at different times has been illustrated in Table 2.

When comparing same time points between the two regimens, significant differences were observed at all times except for the baseline before fluoride rinsing and the baseline before using calcium and fluoride mouthwashes ( $P = 0.070$ ; Table 2).

## DISCUSSION

In the present study, we found that the concentration of salivary fluoride at 1 and 12 hours after rinsing calcium and fluoride mouthwashes was significantly higher compared with the fluoride regimen.

In both regimens, the highest fluoride levels were related to 1 and 12 hours after rinsing and baseline, respectively and they differed significantly.

In the present study, the 1-hour post rinse fluoride value of Ca + F regimen was smaller than an investigation performed by Vogel et al. [6] using 150mmol/lit calcium and 228 ppm fluoride mouth rinses. In evaluating the efficacy of calcium pre-rinse on 1-hour salivary fluoride, Vogel et al. [6] concluded that calcium pre-rinse significantly increased the 1-hour salivary fluoride concentration compared with the control fluoride without a pre-rinse. The increase was almost 9 times higher than the fluoride control mouthwash.

Our results are consistent with these findings and demonstrate the significant effect of calcium pre-rinse on salivary fluoride, by showing the 6.25 fold increase in the 1-hour salivary fluoride after using calcium and fluoride rinses compared to fluoride without calcium pre-rinse. Similar to the study conducted by Vogel et al. [6], we have administered calcium lactate pentahydrate with a comparable concentration; however, this resulted in a lower salivary fluoride concentration 1-hour post rinse. This was despite a higher concentration of fluoride in our mouthwash compared to what Vogel et al. used in their study. This may be contributed to the higher rinsing volume of mouthwash used by Vogel et al.

**Table 1.** Salivary Fluoride Concentration at Baseline, 1 and 12 Hours after Rinsing

	Baseline (F)*	1 h (F)*	12 h (F)*	Baseline (Ca + F)**	1 h (Ca + F)**	12 h (Ca + F)**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Salivary [F] ( $\mu\text{mol/l}$ )	1.201(0.056)	11.744(1.13)	2.520(0.567)	1.145(0.190)	73.417(5.516)	18.711(2.430)

(n= 42). According to repeated measures ANOVA, there was significant difference between fluoride concentrations in different times ( $P < 0.001$ ).

\* Fluoride regimen

\*\* Calcium and fluoride regimen

We found a greater fluoride concentration 12 hours following the use of both mouthwashes compared to investigations performed by Pessan et al. [8], Vogel et al. [10] and Whitford et al. [9]. In two of these studies [8, 9], the impact of calcium rinse before using fluoride dentifrice was assessed. Pessan et al. reported a significant increase in salivary fluoride by using calcium lactate pre-rinse only 1 hour after the use of fluoride dentifrice [8]. However, the effect of calcium pre-rinse was significant at both 1 and 12 hours after rinse in our study. In contrast, Whitford et al. reported that calcium pre-rinse had a very little effect on the fluoride concentration of saliva [9]. Although there are some differences between our study and other studies, including differences in subject population and methodologies, the main difference may be the form of the applied fluoridated product.

In fluoridated dentifrices, there is surfactant sodium lauryl sulfate (SLS) with a strong affinity to bind with calcium and hence could limit the bio-availability of calcium through precipitation of ionic calcium and could affect the fluoride uptake [8].

Furthermore, it may be stated that rinsing the oral cavity with water after using fluoridated dentifrice could wash away some  $\text{CaF}_2$  supplies before they have enough time to stabilize in the oral cavity. Vogel et al. [10] investigated the effect of calcium mouth wash prior using 228 ppm fluoride rinse. In agreement with the study performed by Vogel et al., we have shown that calcium rinse before fluoride rinse increases the 12 hour salivary fluoride content significantly compared with the fluoride rinse alone. Our results are in line with Vogel et al., showing a positive effect of calcium mouth rinse in elevating the 12-hour salivary fluoride level. Furthermore, we found that the amount of 12-hour salivary fluoride was larger than that found in Vogel's study [10]. There was a similar using instruction and calcium mouth rinse concentration in our study and the study conducted by Vogel et al.. Factors such as age disparity in the volunteers, study design, using mouthwashes prepared by different manufacturers, and different washout periods may be factors affecting the increases; of which rinsing with different concentrations of fluoride solution may be the main factor.

**Table 2.** Pairwise Comparison of Fluoride Concentration at Different Times

Time	1 h (F)*	12 h (F)*	Baseline (Ca + F)**	1 h (Ca + F)**	12 h (Ca + F)**
Baseline (F)*	P< 0.001 <sup>a</sup>	P< 0.00 <sup>a</sup>	P= 0.070	P< 0.001 <sup>a</sup>	P< 0.001 <sup>a</sup>
1 h (F)*		P< 0.001 <sup>a</sup>	P< 0.001 <sup>a</sup>	P< 0.001 <sup>a</sup>	P< 0.001 <sup>a</sup>
12 h (F)*			P< 0.001 <sup>a</sup>	P< 0.001 <sup>a</sup>	P< 0.001 <sup>a</sup>
Baseline (Ca + F)**				P< 0.001 <sup>a</sup>	P< 0.001 <sup>a</sup>
1 h (Ca + F)**					P< 0.001 <sup>a</sup>

<sup>a</sup> P < 0.05.

Pairwise differences are based on Least Significant Difference

\* Fluoride regimen

\*\* Calcium and fluoride regimen

In Vogel et al. investigations [6, 10], 20ml fluoride mouth wash was used. It seems that rinsing with 900 ppm fluoride mouth wash in the present study could lead to a greater effect compared to Vogel et al. [10] study using 228 ppm fluoride mouth rinse on 12-hour post rinse salivary fluoride. However, compared to Vogel et al. [6] we did not find such an effect 1-hour after rinsing, and only the greater volume of rinsing solution could explain the difference between our and Vogel et al studies.

The soft tissue fluoride reservoirs seem to be a source of salivary fluoride after the application of topical fluoridated products [10]. So considering the certain role of calcium-mediated fluoride supplies, the respective 6.25 and 7.42 fold increases in 1 and 12 hours fluoride concentrations found in our study compared with when the pre-rinse was not used may be associated with the increased formation of soft tissue calcium-mediated fluoride reservoirs as a result of using calcium pre-rinse before fluoride rinse. This suggests that a huge elevation in salivary fluoride produced by calcium pre-rinse may induce a greater cariostatic effect. One of the advantages of calcium pre-rinse before fluoride rinse is that the need to concentrate the fluoride amount of the used product to maximize the salivary fluoride is reduced. Therefore, the risk of accidental swallowing of fluoride is decreased. In addition, the taste of calcium fluoride is not bitter and is suitable for users.

Although following increased salivary fluoride concentration, the likelihood of formation of fluoride reservoirs in the plaque phase is improved [10], it should be kept in mind that the higher concentration of salivary fluoride after using both calcium and fluoride rinses found in our study, does not completely reflect the concentration of this ion at the surface of the enamel. Such concentration is related to the demineralization and remineralization process [3].

It should also be emphasized that although children and adolescents are the major group of beneficiaries of preventive measures, adults also benefit.

Because adults are retaining their teeth longer than in previous decades, the impact of salivary fluoride on the teeth of adults has become more important [11]. Another reason for choosing adult population in our study was that we were able to exert interventions and control the exact time of sampling in this group. In addition, all these participants had similar conditions such as diet. The effect of gender has also been deleted. In our opinion, no other statistical population could be controlled as accurate as our participants. Inability to measure the calcium concentration was the limitation of our study, therefore an investigation in a cross-over manner, in which the salivary and plaque calcium and fluoride concentration is assessed is recommended. Moreover, from the community-based point of view, although calcium fluoride is low price, determining the cost effectiveness of this pre-rinsing with before and after studies is suggested.

## **CONCLUSION**

Prescribing calcium before fluoride rinse enhances the anti-caries effectiveness of fluoridated rinses. We recommend calcium pre-rinsing before 900 ppm fluoride rinse.

## **ACKNOWLEDGMENTS**

This manuscript was based on a thesis (registration code: 2143) submitted to the graduate faculty, Faculty of Dentistry, Zahedan University of Medical Sciences, in partial fulfillment of the requirements for the M.S. degree. The authors would like to thank the Vice Chancellor for Research of Zahedan University of Medical Sciences for their financial support.

## **REFERENCES**

1- Tschoppe P, Siegel A, Meyer-Lueckel H. Saliva substitutes in combination with highly

- concentrated fluorides and brushing: in vitro effects on enamel subsurface lesions. *Caries Res.* 2010;44(6):571-8.
- 2- Whitford GM, Wasdin JL, Schafer TE, Adair SM. Plaque fluoride concentrations are dependent on plaque calcium concentrations. *Caries Res.* 2002 Jul-Aug;36(4):256-65.
- 3- Vogel GL, Mao Y, Chow LC, Proskin HM. Fluoride in plaque fluid, plaque, and saliva measured for 2 hours after a sodium fluoride monofluorophosphate rinse. *Caries Res.* 2000 Sep-Oct;34(5):404-11.
- 4- Rosin-Grget K, Sutej I, Lincir I. The effect of saliva on the formation of KOH-soluble fluoride after topical application of amine fluoride solutions of varying fluoride concentration and pH. *Caries Res.* 2007;41(3):235-8.
- 5- Ogaard B. CaF<sub>2</sub> formation: cariostatic properties and factors of enhancing the effect. *Caries Res.* 2001;35 Suppl 1:40-4.
- 6- Vogel GL, Chow LC, Carey CM, Schumacher GE, Takagi S. Effect of a calcium prerinse on salivary fluoride after a 228-ppm fluoride rinse. *Caries Res.* 2006;40(2):178-80.
- 7- Lagerlöf F, Ekstrand J, Rölla G. Effect of fluoride addition on ionized calcium in salivary sediment and in saliva. *Scand J Dent Res.* 1988 Oct;96(5):399-404.
- 8- Pessan JP, Sicca CM, de Souza TS, da Silva SM, Whitford GM, Buzalaf MA. Fluoride concentrations in dental plaque and saliva after the use of a fluoride dentifrice preceded by a calcium lactate rinse. *Eur J Oral Sci.* 2006 Dec;114(6):489-93.
- 9- Whitford GM, Buzalaf MAR, Bijella MFB, Waller JL. Plaque fluoride concentrations in a community without water fluoridation: effects of calcium and use of a fluoride or placebo dentifrice. *Caries Res.* 2005 Mar;39(2):100-7.
- 10- Vogel GL, Chow LC, Carey CM. Calcium pre-rinse greatly increases overnight salivary fluoride after a 228 ppm fluoride rinse. *Caries Res.* 2008;42(5):401-4.
- 11- Harris NO, Garcia-Godoy F, Nielson CN. *Primary Preventive Dentistry.* 7th ed. London: Pearson; 2009.