THE EFFECT OF A CALCIUM PRE-RINSE ON THE SALIVARY AND PLAQUE FLUORIDE AND CALCIUM CONCENTRATIONS AFTER A 900 PPM NaF RINSE: A PLACEBO-CONTROLLED RANDOMIZED CLINICAL TRIAL

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ABSTRACT: The amount of available calcium delivered to plaque is dependent on the use of calcium compounds. This study assessed the salivary and plaque concentrations of the fluoride ion [F] and the calcium ion [Ca] after the use of a calcium pre-rinse. Thirty subjects completed this double-blind crossover trial in a dormitory in Zahedan, Iran. The subjects were recruited using a table of random numbers. After prophylaxis and two weeks of oral hygiene practices using non-fluoride products, 15 subjects, chosen randomly, rinsed with calcium placebo/sodium fluoride and the other 15 subjects rinsed with calcium lactate/sodium fluoride. One and 12 hours later, salivary and plaque samples were collected. After another prophylaxis and the elapse of a further two weeks, with the previous conditions of oral hygiene using non-fluoride products, the participants rinsed with the treatment that they had not previously used. After 1 and 12 hours, sampling was again done. The [F] and [Ca] were analyzed using a fluoride electrode and atomic absorption spectrometry, respectively. The data were analyzed using repeated measures ANOVA at a significance level of 5%. The 1 and 12 hour salivary [F] were significantly higher after the calcium and fluoride rinses ($p<0.001$). No significant difference was found in the plaque [F] between the two rinse treatments ($p>0.05$). A significant difference was detected in the plaque [F] when comparing the values obtained at different times with each treatment ($p<0.001$). With respect to the salivary and plaque [Ca], there was no significant difference in the values obtained at the different times for each treatment, regardless of whether the rinse was with calcium or calcium placebo ($p>0.05$). The salivary [F] increased with the calcium and fluoride rinsing. Our attempts to increase the salivary [Ca] and plaque [F] and [Ca], by using a calcium pre-rinse met with no success.

Keywords: Calcium fluoride; Calcium lactate; Dental plaque; Mouthwashes; Saliva; Sodium Fluoride.

INTRODUCTION

Dental caries is the most prevalent infectious disease in the general population.[1-3] Untreated caries can affect different aspects of growth and development.[4,5] The widespread use of the fluoride ion (F) has been considered to be proven factor in the reduction of dental caries.[6-10] For a long time, it has been recognized that the cariostatic activity of F is mainly through its topical post-eruptive effect.[6,11,12] At present, it is known that the beneficial sharply elevated salivary and plaque [F] observed shortly after the use of a fluoride agent falls rapidly back to the baseline level within 2–4 hr.[13,14] By considering the fact that the diet is the main source of F intake,[15-19] the WHO emphasizes the repetitive and regular use of home care low concentration fluoridated products.[12, 20] In this regard, F mouthwashes are important home care products.[21,22]

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The concentration of F in the fluid bathing the teeth is profoundly maintained by the diffusion of this ion from CaF_2 deposits, the main product of topical fluoride application.[11,23,24] It now appears certain that CaF_2 deposits act as significant F reservoirs, either in the form of mineral or in association with a biological material.[24-26] CaF_2 serves as a long term (two week) reservoir of the fluoride ion as a deposit on the tooth surface, plaque, and mucosa.[20,27,28]

The mechanisms of action of F are greatly dependent on the F concentration in whole saliva, which is the vehicle for delivering F to the dental plaque, and, especially, on the F concentration in the dental plaque itself.[14] On the other hand, it is well documented that the plaque calcium concentration is of great importance in the uptake and retention of F by dental plaque.[11,29] Calcium availability is considered to be one of the major factors responsible for the formation of CaF_2.[23] Calcium ions can be released from enamel, (resulting in a concentration of 0.02 mmol/L), and, most notably, from saliva and dental plaque fluid (resulting in a concentration of 1–3 mmol/L).[23,30]

Considering these facts, during F usage the formation of these reservoirs is limited by the low concentration of unbound oral calcium in the oral fluids (1 mmol/L), relative to the amount of topically applied F (12–48 mmol/L).[24,27] Calcium lactate solution is one of several calcium compound vehicles that can deliver calcium to dental plaque.[29]

Previous attempts have focused on approaches to overcome this limitation by increasing the plaque calcium concentration and hence facilitate the precipitation of CaF_2. [11,26,27,30] An increase in the overnight salivary [F] was reported by Vogel et al. using a calcium lactate pre-rinse prior to the use of a 228 ppm F rinse.[31] In another study on the 1 hr salivary [F], Vogel et al. reported that a calcium pre-rinse given immediately before a 228 ppm F mouth rinse resulted in a significantly increased [F].[27] In further study by Vogel et al. on the 1 hr plaque [F], a calcium pre-rinse led to a significantly increased [F] after a F rinse.[26] In a study conducted by Furlani et al. on the plaque [F] and [Ca], the use of a calcium lactate rinse prior to the use of a F dentifrice was able to significantly increase the level of both elements.[11] Heijnsbroek et al. demonstrated that an increased salivary [F] after post-brushing F rinsing did not lead to an increased [F] in the newly formed (6 hr) plaque.[13]

Despite the studies mentioned above, evaluating the 1 hr and 12 hr salivary and plaque [F] and [Ca] after the use of a fluoride dentifrice preceded by a calcium rinse, Pessan et al. found no significant effect on plaque [F], but a significant increase in salivary [F] at only 1 hr after the use of pre-rinse/fluoridated dentifrice. This combined treatment did not have any effect on the salivary and plaque [Ca].[29] Whitford et al., however, have presented data suggesting that rinsing with a calcium chloride solution immediately before using a fluoridated dentifrice resulted in only a minor impact on the [F] and [Ca] of the saliva and no impact on the plaque contents.[32]

Previously, we have conducted a trial pertaining to the impact of a calcium lactate pre-rinse on the 1 hr and 12 hr salivary [F] after a 900 ppm fluoride mouth rinse. The results led to the conclusion that the salivary [F] was significantly higher with the pre-rinse at both times after the use of a fluoridated mouthwash.[20]

In Iran, a 900 ppm F mouthwash is currently marketed as a home care product that can be self-applied. Moreover, it has been used in school-based programs once a week. Fluoride rinse systems are used to enhance the effectiveness of topical F and since no studies have been made of the simultaneous concentrations of the fluoride and calcium ions after the use of a calcium lactate pre-rinse and a 900 ppm F mouthwash, the purpose of the present study was to evaluate the F and calcium concentrations in the saliva and dental plaque after the use of a 900 ppm F mouthwash preceded by a calcium lactate rinse.

MATERIALS AND METHODS

In this double-blind, double-crossover clinical trial (Iranian Registry of Clinical Trial ID No: IRCT201404286105N3), a sample of 30 Iranian dental students, ranging in age from 18 to 24 years were recruited as the subjects. They were residents of the boys' dormitory of the Zahedan University of Medical Sciences (ZUMS) in the south east of Iran, which has one filtered water supply. This sample selection allowed the investigator greater access for baseline and follow-up evaluations.

The Research and Ethics Committee of ZUMS viewed and approved the study protocol which adhered completely to the Declaration of Helsinki (ethics code: 6318).The nature and objectives of the study and also the necessity to strictly adhere to the survey protocol throughout the study were explained verbally and in writing to each subject. If they agreed to participate, they signed an informed consent form. A sample size of 30 subjects was determined, based on 90% power and a type I error of 0.05. Out of 63 students, 45 individuals met the inclusion criteria and were to willing to participate. From these 45 persons, 30 subjects were selected for the study using a table of random numbers (Figure 1).

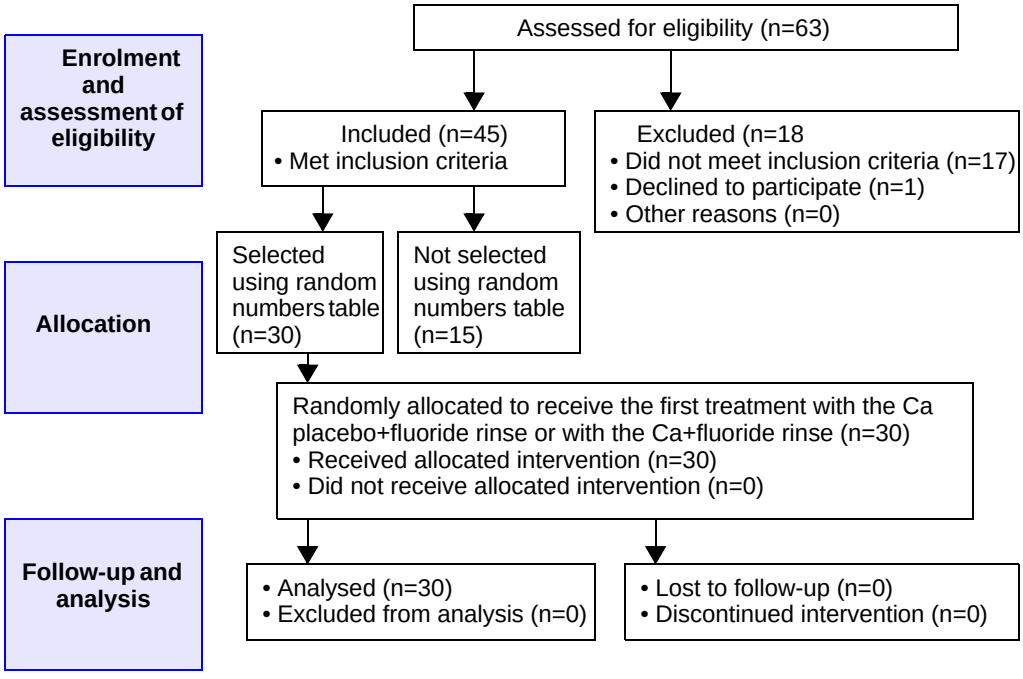

Figure 1. Participants' flow diagram.

The inclusion criteria for the subjects: These included: good oral hygiene which was checked clinically by the absence of food debris and heavy plaque accumulations on the teeth, at least 24 natural teeth, a normal unstimulated whole saliva flow rate (0.25–0.35 mL/min), good oral health (no demineralized enamel or untreated decay) and general health, no dentures, orthodontic appliances, or faulty dental restorations, and no medications for at least 4 weeks before the project started.

The mouthwashes used in the study: These were sodium fluoride (900 ppm, Behsa, Iran), 160 mmol/L calcium lactate (Merck, Germany), and calcium placebo (deionized water, Iran). The rinses were poured into identical containers and delivered to the subjects by a trained individual who had been employed. The containers were identified only by a code, so that neither the direct clinical investigator nor the volunteers were aware of whether a rinse contained fluoride, calcium, or calcium placebo. Similarly, the analyst was also blinded as to the identity of the test specimens.

Phases of the study: This study was performed over 28 days in 4 different phases:

(i) Preparation phase (1^{st}–14^{th} day): At the beginning, dental prophylaxis was performed for all the volunteers in a dental school in order to remove plaque so as to enable the experiment to be started under similar conditions for all the subjects. Afterwards, the volunteers were asked to do their daily oral hygiene practices for the following 14 days using non-fluoride containing dental products. However, to allow some dental plaque accumulation, the subjects were instructed to avoid tooth brushing in last 2 days of this phase. They were also asked to refrain from drinking tea, a drink with a high F content, on the day before the ending this phase. On the 14^{th} day, in the dormitory 1 hr after serving dinner, the first baseline salivary and plaque sampling were done at 7–8 pm. Unstimulated whole saliva was expectorated for 2 min into an empty plastic vial in a resting and upright position. By considering the volume of the gathered saliva, the flow rate was calculated. Subsequently, plaque samples were collected from different surfaces of all first and second molars and premolars using a sterile curette with gentle pressure and transferred to an empty plastic tube.

(ii) Rinsing phase (at the end of 14^{th} day): Immediately after the first baseline sampling, using a simple randomization, half of the subjects were given the 10 mL calcium lactate rinse/10 mL sodium fluoride rinse treatment and the other half were given the 10 mL calcium placebo rinse/10 mL sodium fluoride rinse treatment. The subjects then rinsed with each of the volumes provided for 1 min in the same order, as written above, without any interruption between the mouthwashes. The subjects were asked not to consume foods of beverages, except for water, or follow any oral hygiene practices, etc., during the following 12 hr until the subsequent samplings had been done at 7–8 am the following morning. Thus two lots of sampling of saliva and plaque were done: one at 7–8 pm and the other 12 hr later at 7–8 am.

(iii) Washout phase (14^{th}–28^{th} day): The subjects then returned to the clinic to receive another prophylaxis. They then adhered to 2 weeks of performing their daily oral hygiene practices using non-fluoride containing dental products, with the avoidance of brushing in the last 2 days, and the avoidance of drinking tea on the last day, as was done in the preparation phase. At the end of this phase, the second

baseline salivary and plaque samples were gathered, at 7–8 pm, under the conditions described for the preparation phase.

(iv) Crossover rinsing phase (at the end of 28th day): After end of the washout period, the participants were given the rinsing treatment that they had not previously used in the rinsing phase (crossover design). The volume of the rinses, the time of rinsing, the order of using the rinses, the duration of the rinsing, and the timing and conditions of the salivary and plaque sampling (the 1 hr and 12 hr samples) were the same as were used at the end of the 14th day.

Throughout the study, the sequences of delivered foods, fruits, drinks, etc., served in this dormitory were stable and repeated weekly so that everything at the 2 phases of rinsing and crossover rinsing was the same. Moreover, during the hours spent outside the dormitory, the participants carried and drank from bottles filled with dormitory water and did not drink any other water.

A dental student, who lived in the dormitory recruited the participants according to the inclusion criteria, assigned the participants to the two groups, and supervised them.

The techniques used by Vogel et al.[17] and Whitford et al.[18] were used to prepare the saliva and plaque samples, respectively. The fluoride and calcium in the prepared specimens were then analyzed using an inverted fluoride selective electrode apparatus (Metrohm Co, Swiss) and atomic absorption spectrometry (Pars Azmun Kit). The outcomes are presented as mean±standard deviation. All variable data were normally distributed so the repeated measures ANOVA in the SPSS 18 for Windows (SPSS Inc., Chicago, Illinois, USA) were used. A significance level of 5% was set. The time of the post-rinse sampling and the mouth rinse regimen were two factors considered for data analysis. Pairwise comparisons were based on the Least Significant Difference.

RESULTS

Recruitment was done in January 2014, all the subjects (age range 18–22 years, mean age 21±1.4 years) were studied simultaneously, and they all completed the trial (Figure 1). Table 1 represents the average [F] and [Ca] of saliva and dental plaque before, 1 hr after, and 12 hr after the use of the fluoride rinse, preceded by the use of either the calcium placebo rinse or the calcium rinses, as well as the number of samples studied. Regardless of the rinsing treatment used, the results showed no statistically significant differences in the salivary flow rates and the plaque sample dry weights at the different times ($p>0.05$). There were also no significant differences in the first and second baseline concentration values (before each treatment) for all the studied variables ($p>0.05$). Therefore, the differences among the salivary and plaque [F] and [Ca] were due to the variable of the calcium pre-rinse given and not to any differences in flow rates, plaque weights, or baseline values.

Regarding the mean salivary [F], significant differences were revealed between the different time points ($p<0.001$) and also in all the pairwise comparisons ($p<0.001$). The mean 1 hr post-rinse salivary [F] were significantly higher than the 12 hr post-rinse value with the calcium placebo and fluoride rinse treatments as well as with the combination of the calcium lactate pre-rinse and fluoride rinse ($p<0.001$). Comparing the corresponding times elapsed after each treatment, statistical analysis detected a

significant difference in favor of using the calcium pre-rinse and fluoride rinse treatment ($p<0.001$). The calcium and fluoride rinses increased the [F] by nearly 6.5 and 8 times compared to the calcium placebo and fluoride rinse treatments at 1 hr and 12 hr respectively.

Table 1. One hr and 12 hr post-rinsing salivary and plaque concentrations of fluoride [F] and calcium [Ca]. (Values are mean±SD, n=size of the sample)

Variable (n)	First baseline	Calcium placebo and fluoride rinse treatment		Second baseline	Calcium and fluoride rinse treatment	
		1 hr	12 hr		1 hr	12 hr
Salivary [F] (µmol/L) (n=30)	1.31 ±0.27	10.41 ±0.75*	2.08 ±0.29	1.42 ±0.26	67.79 ±2.28*	16.19 ±0.79
Plaque [F] (mmol/kg) (n=30)	1.96 ±0.18	10.84 ±0.56*	2.02 ±0.34	2.06 ±0.36	10.53 ±0.50*	1.99 ±0.31
Salivary [Ca] (mmol/L) (n=30)	1.53 ±0.34	2.55 ±0.56	2.57 ±0.25	1.65 ±0.43	2.57 ±0.14	2.51 ±0.25
Plaque [Ca] (mmol/kg) (n=30)	278 ±98	305 ±85	301 ±32	269 ±92	321 ±118	300 ±96

*Compared to the 12 hr value for the the same rinse treatment $p<0.001$.

The use of either the calcium pre-rinse or the calcium placebo pre-rinse produced no significant difference in the mean plaque [F] at the same post-rinse time periods ($p>0.05$). Statistically significant differences were found, however, when the mean values at 1 hr and 12 hr were compared. The mean plaque [F] was significantly higher at 1 hr than at 12 hr with both treatments ($p<0.001$).

No significant differences were detected between the 1 hr and 12 hr salivary and plaque [Ca] with each treatment ($p>0.05$). There was also no significant differences between the same post-rinse times with the two treatments ($p>0.05$). The pairwise comparisons of the studied variables are shown in Table 2.

DISCUSSION

Even under the best possible conditions, the complete removal of dental plaque is impossible. Hence, the concentration of plaque-incorporated compounds, especially fluoride, plays a critical role against dental caries.[29] We designed this study protocol and performed it with the ultimate purpose of increasing the plaque [F]. As mentioned in introduction, other researchers studied the effects of different calcium compounds and fluoride products as well as different concentrations of calcium and fluoride solutions on salivary and plaque [F] and [Ca].[14,20,26,27,29,31,32]

In the current study, the salivary [F] values were significantly higher after the calcium and fluoride rinses at both the 1 hr and the 12 hr time points. The plaque [F] was significantly increased at 1 hr after rinsing with or without the calcium pre-rinse compared to the samples collected at 12 hr. However, the salivary and plaque [Ca]

after the use of the calcium and fluoride rinses were not significantly different from those observed after the use of the calcium placebo and fluoride rinses. There were also no significant differences in the salivary [Ca] or the plaque [Ca] at 1 hr and 12 hr post-rinse.

Table 2. Pairwise comparison of the salivary and plaque [F] and [Ca]

Variable	Time	p values		
		12 hr (Placebo+F)	1 hr (Ca+F)	12 hr (Ca+F)
Salivary [F]	1 hr (Placebo+F)	<0.001	<0.001	<0.001
	12 hr (Placebo+F)	----------	<0.001	<0.001
	1 hr (Ca+F)	----------	----------	<0.001
Plaque [F]	1 hr (Placebo+F)	<0.001	0.071	<0.001
	12 hr (Placebo+F)	----------	<0.001	0.781
	1 hr (Ca+F)	----------	----------	<0.001
Salivary [Ca]	1 hr (Placebo+F)	0.879	0.852	0.647
	12 hr (Placebo+F)	----------	0.985	0.345
	1 hr (Ca+F)	----------	----------	0.236
Plaque [Ca]	1 hr (Placebo+F)	0.808	0.570	0.858
	12 hr (Placebo+F)	----------	0.416	0.967
	1 hr (Ca+F)	----------	----------	0.472

Our findings indicate that the calcium lactate pre-rinse significantly increased the salivary [F]. Compared the values obtained with the calcium placebo pre-rinse, the use of the calcium lactate pre-rinse lead to an increase in the 1 hr and 12 hr salivary [F] of nearly 6.5 and 8 times, respectively. The present findings are in agreement with a previous study by Ramazani et al, which tested only the salivary [F], involving the use of a 150 mmol/L calcium lactate pre-rinse immediately before rinsing with a 900 ppm NaF rinse.[20] Although the amount of salivary [F] was uniformly higher in that study,[20] the present study supports the same conclusion. The authors confirmed their previous finding of a highly significant increase in the salivary fluoride concentration after calcium pre-rinsing. It should be noted that the present study protocol was the same as that used in our previous study (i.e., both studies had the same duration of washout period, crossover design, age range of subjects, and concentration of the fluoride and calcium rinses). There were some differences, however, such as in the

sample size and the gender of the participants. The main difference was the inclusion of a calcium placebo rinse. We used calcium placebo so as to be able to include two rinses in each treatment. The subjects were therefore blinded as to the type of treatment they were using.

In their study, Whitford et al. concluded that 20 mmol/L calcium chloride pre-rinse did not have any significant impact on both the 1 hr and 12 hr salivary [F] elevation after using a 1074 mg F/kg NaF dentifrice.[32] Meanwhile, Vogel et al. found that 150 mmol/L calcium lactate pre-rinse given before a 228 ppm NaF rinse had a substantial effect on the 1 hr salivary [F].[27] Pessan et al. compared the salivary [F] after the use of a 1030 ppm NaF dentifrice preceded by a 150 mmol/L calcium lactate rinse. In that study, a large increase in the value of the salivary [F] was reported only at 1 hr after the calcium pre-rinse/fluoride dentifrice.[29] In another study, Vogel et al. determined the 12 hr salivary [F] after rinsing with a 228 ppm NaF solution. They observed a significant increase in the salivary [F] in the 12 hr samples after the use of calcium and fluoride rinses compared to the use of a control NaF rinse.[31] The values reported in these previous investigations are somewhat larger or smaller than those obtained in the current study. However, what is noteworthy in these studies is that our finding, of significant salivary [F] increases after the use of a pre-rinse and a F rinse, was observed in the studies that used a pre-rinse before a fluoride rinse but not when the pre-rinse was used before a fluoridated dentifrice. The main reason for the difference in the results of our studies and the studies of dentifrices is the existence of calcium-binding surfactants, particularly sodium lauryl sulfate, in dentifrice and the use of post-brushing water rinsing, both of which decrease the amount of calcium available to react with fluoride. This surfactant has a strong affinity for calcium and precipitates some of the calcium delivered by the calcium pre-rinse.[29,31]

However, although there is strong evidence that the increase in salivary [F] is a prerequisite of the cariostatic effect, the medium which is most relevant to the histologic changes of the caries process is none other than the dental plaque. Hence, we do not expect that a large increase in the salivary [F] can induce an anticaries effect on its own. The only conclusion that can be drawn is that, in order to get an increase in the salivary [F] great enough to form sufficient calcium fluoride deposits to affect the caries process, a high amount of calcium is needed.

The plaque [F] at 1 hr as well as at 12 hr after each rinsing treatment was not significantly different. Moreover, the values present at 1 hr post-rinse were significantly higher than those at 12 hr. Some studies have evaluated the amount of fluoride in dental plaque by testing different fluoride and calcium treatment regimens.[29,32] In comparing the values obtained with the different treatments and different post-rinse times, our conclusions are in agreement with those of the studies by Pessan et al.[29] and Whitford et al.[32] In the investigation conducted by Whitford et al., no effect was found on the 1 hr and 12 hr plaque sample [F] after using a calcium chloride pre-rinse and then a NaF dentifrice.[32] Using a calcium lactate pre-rinse, Pessan et al, also concluded that the plaque [F] was not affected by using a fluoride dentifrice.[29] Our mean values were relatively larger or smaller than those in the aforementioned studies. We found a significantly higher 1 hr plaque [F] which is consistent with the results of the last two author groups and consider that nearly

almost all of the fluoride in the fluoride-saturated plaque will be discharged within a short period.

More recently, in another paper by Vogel et al., the effect of a calcium pre-rinse on plaque [F] after a F rinse was reported. The authors demonstrated that a 228 ppm NaF rinse preceded by a 150 mmol/L calcium lactate rinse increased the 1 hr post-rinse plaque [F] nearly 12 times compared to when the F rinse was used without a calcium pre-rinse.[26] Generally, this study by Vogel et al. is similar to our study in its basic methodology but differs somewhat in the details. We used a 900 ppm NaF rinse which has four times more F than those that Vogel et al. tested. The question may be asked as to why, in spite of the higher concentration of F in used in our F rinse, the mean 1 hr post-rinse plaque [F] with the calcium and fluoride rinse treatments was lower in the current study compared with the same treatment in the study of Vogel et al.? There is one possible answer. During the rinsing with a more concentrated F solution, the salivary [F] greatly increases in comparison to the salivary [Ca], as occurred in our study. As a consequence, the formation of more calcium fluoride deposits is induced. The likelihood of the migration of these compounds into the plaque structure is low and instead they may be swallowed or expectorated. Calcium fluoride might also rapidly accumulate on the plaque surface and make a barrier which would restrict the penetration and uptake of fluoride into the dental plaque.[29,32] The chance of occurrence of these mentioned events would be less with the low fluoride concentrated solutions which would promote the penetration of free fluoride into the interior of the plaque.

It also raises a great interesting question about the non-significant difference of plaque [F] associated with the two treatments (with or without pre-rinse), despite of higher salivary [F] in the pre-rinse included treatment. It is possible that, following the fluoride rinsing, most of the fluoride migrated into the plaque or accumulated on its surface and then, as the F diffused back into the saliva, the plaque [F] declined.[14,29] Hence, it is thought that the increase in the pre-rinse associated plaque [F] is reversible. In other words, the more F is present in saliva, the greater is the chance that F will return to the saliva from the plaque. As a result, no significant difference was found in the plaque [F], at the corresponding times, with the two treatments, with and without a calcium pre-rinse.

In contrast to the findings for the salivary [F], there was no significant difference in salivary [Ca]. Consistent with our findings, a study by Pessan et al., found a similar result of no significant increase in the salivary [Ca] after the use of a fluoride dentifrice, with or without the use of a calcium lactate pre-rinse.[29] In another study, by Whitford et al., the use of a calcium rinse had only a minimal effect on the salivary [Ca].[32] It is not known why the salivary [Ca] did not increase with use of a calcium pre-rinse.

In our study, using calcium lactate as a pre-rinse followed immediately by the use of a fluoride rinse did not have a substantial impact on the plaque [Ca]. The failure to increase the plaque [Ca] might be interpreted as follows: the calcium compounds formed after the use of the calcium and fluoride rinses released calcium into the dental plaque only very slowly due to their low solubility.[32] In both our study and in a study by Vogel et al.,[26] no significant increase in the plaque [Ca] occurred with the calcium and fluoride rinse treatments.

For comparison, in a previously reported investigation,[14] the range of the plaque [Ca] was relatively narrow (102–518 mmol/kg dry weight). There is a growing body of evidence which supports the view that saliva is the main contributor to the plaque calcium.[14,32] However, due to presence of little variance among the subjects in the salivary [Ca], the observed range of the plaque [Ca] may be due to variations in the composition of the plaque itself.

There is one limitation that must be taken into account. In our survey, we collected the samples after only a single usage of each treatment. We were, however, aware of the fact that 1–2 weeks need to pass for the F reservoirs to be filled. If the rinsing treatments were given for a relatively long period, it would be impossible to restrict the subjects to the use of only our two administered treatments and for them to avoid the use of other fluoride products. Thorough supervision for a longer period would also be more difficult. If the rinsing had been continued for 7–14 days before the samples were collected, the confounding effects of many factors were likely to have arisen. Therefore, the likelihood of this confounding occurring was minimized by sampling after one use of each of the two treatments.

An analysis of the study methodology also revealed a strength. Ramazani et al. emphasized that during the period of non-fluoride product usage the fluoride reservoirs took nearly two weeks to be eliminated.[20] For this reason, we separated the two stages of the study with a washout period of two weeks in order to eliminate the confounding effect of one treatment on the other one.

CONCLUSION

Using 150 mmol/L calcium lactate rinse immediately followed by a 900 ppm fluoride rinse significantly increased the salivary [F]. However, the calcium and fluoride rinse treatments had no effect on the salivary [Ca] and the plaque [F] and [Ca]. Although we can generalize our findings to an adult population, further studies on populations with a lower age are recommended. Future studies should focus on using a modified design to enhance the plaque [F]. Studies using different concentrations of the calcium pre-rinse appear promising for achieving this ultimate purpose.

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DECLARATION OF INTERESTS

The authors report no personal or financial interest in the products or companies described in this article.

OTHER INFORMATION

Trial registration: Name of trial: Comparison of calcium and calcium placebo pre-rinse effect on salivary and plaque fluoride and calcium concentrations after the use of 900 ppm sodium fluoride mouthwash

The full trial protocol is available from: http://www.irct.ir,

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