Reducing Microbial Levels in High Caries Risk Adults – Randomized Clinical Trial

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Abstract

Background: Dental caries is an infectious transmissible chronic disease. Unless the microorganisms initiating decalcification of the enamel are dealt with initially, the restorative management of dental caries is doomed to failure.

Objective: The purpose of this study was to determine if a NaOCl-povidone-I2 rinse was more effective than a povidone-I2 rinse alone in decreasing microbial levels in high caries risk adults.

Methods: Forty-eight participants were examined to determine their caries experience and randomized into treatment (TX) and control (CT) groups. At baseline, each participant gave a saliva sample for the CRT® test and a plaque sample for the CariScreen® test.. The TX group rinsed with 15 ml of 1.6% NaOCl (The Clorox Company, Oakland, CA, USA) for one minute followed by rinsing with 15 ml of 10% povidone-I2 (Betadine®. Aviro HealthLP, Stamford, CT. USA) for one minute. The CT group rinsed with 15 ml of povidone-I2 for one minute. The CRT® and CariScreen® tests were repeated at one, four, eight and twelve weeks.

Results: The CRT® test showed that the TX group kept the level of Streptococcus mutans lower than the CT group at four weeks and through the remainder of the study. The CRT® test showed the TX group kept the level of Streptococcus mutans lower than the CT group at four weeks and for the remainder of the study. The CariScreen® test showed that both groups kept organisms low for only one week.

Conclusion: The use of NaOCl before povidone-I_2 (TX) did enhance the effect of the povidone-I_2 . The CariScreen® readings were low at week one, but increased rapidly hereafter suggesting inaccurate readings.

Keywords: CAMBRA, povidone-iodine, Sodium hypochlorite, CRT® test, CariScreen® test

Introduction

The purpose of this study was to determine if a NaOCl-povidone-I_2 rinse (TX) was more effective than a povidone-I_2 rinse (CT) in decreasing microbial levels in high caries risk adults. The hypothesis was the TX mouth rinse will be more effective in lowering the microbial counts than the CT rinse for twelve weeks. Even with the decreasing trend in dental caries experience for adults 20-64 years of age (25%), the prevalence of untreated tooth decay remains high (44%) for adults living at less than 100% of the Federal Poverty Level (FPL) [1]. Dental caries is a transmissible infectious disease [2]. Surgical repair of the cavitated tooth without eliminating the cause is illogical. Decreasing the level of *Streptococcus mutans (S. mutans),* Lactobacillus and cariogenic microorganisms are imperative before applying fluoride agents [3]. The difficulty, however, is penetrating the biofilm on the enamel where the *S. mutans* and Lactobacillus reside with a topical antimicrobial agent. Iodine (10% povidone-iodine) has been demonstrated to effectively reduce *Streptococcus mutans* and lactobacilli for up to three months in children two to six years of age [4]. The use of topical povidone-iodine in adults has been less promising and inconsistent in reducing mutans streptococci and lactobacilli [5]. NaOCl has been used in dentistry for many years. Like povidone-I_2 it is a bactericidal that has been used in endodontics as a disinfectant, in mouth rinses [6] and in periodontal pockets [7]. Both povidone-I_2 and NaOCl kill microrganisms by rapid bactericidal action [8]. NaOCl is a strong oxidizing agent that inhibits enzymatic action within bacterial cell walls. Povidone-I_2 is also a strong oxidizing agent that interferes with fatty acids in the cell walls of bacteria. (8). Both agents are relatively inexpensive.

Materials and methods

Study Population. The participants were recruited from 2010 Census data. The study commenced in May 2011 and finished in February 2012. After receiving approval from the Intuitional Review Board (IRB) on the Human Subject Consent Form, the study commenced on May 2011 and finished on February 2012. The 2010 Census data revealed a population of 21,115 with a median age of 45 years. Approximately 47% were males and 53% were females. Whites made up approximately 78% of the population, followed by Latinos 27% and Asians 11%. The average income per household was $101,738 and unemployment 5.35% [9].

Study Sample. The participants for this study received information about the study from fliers that were placed at a community supported dental clinic. The dental clinic is dedicated to treating only children and is operated by the local assistance league. Therefore, only parents and caretakers of children attending the clinic received information about the study, which spread by word of mouth throughout the community. An initial screening was conducted by the receptionist, either in person or via the telephone, asking questions about disease indicators and risk factors in the Caries Management by Risk Assessment (CAMBRA) protocol (10).7 Participants who answered favorably to all of the disease indicators and risk-factors were given an appointment for an intraoral examination. The participants received $50 and an Oral-B Vitality brush if they attended all five appointments or $10 per appointment.

Prior to conducting the intraoral examination, participants signed an IRB approved consent form (# 10-000891) which included the following *inclusion* and *exclusion* criteria. The ***inclusion criteria*** included: being over 20 years of age; having at least one obviously cavitated tooth; refraining from

smoking, brushing the teeth and using mouthwash the day of the bacterial sampling; refraining from using a commercial mouth rinse during the 12 weeks of the study; having any two of the following risk factors in the CAMBRA protocol which qualifies as a high caries risk [10]. The caries *disease indicators* and *risk factors* included: receiving fillings within the past three years; snacking frequently between meals; presenting with hyposalivation due to medication, radiation or systemic conditions; visual presence of heavy plaque accumulation; and a minimum of 20 natural teeth (not a risk factor).

The CAMBRA protocol also includes caries risk factors such as exposed roots (gingival recession), deep pits and fissures or developmental defects, interproximal enamel lesions/radiolucency, enamel white spot lesions or occlusal discoloration, and recreational drug use. Typically, any person with disease indicators will invariably have some of the moderate caries risk factors. The exclusion criteria included: using a systemic antibiotic within the past three months; currently receiving dental treatment or planning to receive treatment within the next 12 weeks (emergency treatment allowed); being pregnant or nursing; any thyroid disease or sensitivity to iodine; the use of a commercially available mouth rinse the day of the screening examination; smoking, brushing the teeth and using a mouth rinse the day of the bacterial sampling; not being able to make morning examinations.

Intra-oral Examination. All of the dental examinations were conducted in a dental operatory with air, water and light available. All protective barrier methods were used in examining participants with presterilized and disposable instruments. One dentist performed all the clinical examinations. Kappa values to determine intra-examinator reliability were 0.8 to 0.9 for caries experience.

Microbial tests. The CRT® Bacterial Test uses a selective culture media for S. mutans and Lactobacillus. The CariScreen® Caries Susceptibility Test measures adenosine triphosphate (ATP). When ATP is brought into contact with the liquid-stable luciferase/luciferin reagent within the CariScreen® Swab, used to obtain a plaque sample , light is emitted in direct proportion to the amount of ATP present. The CariScreen® meter measures the amount of light generated and provides information about the levels of bacteria present in the biofilm in real-time [11].

Sequence of the examination/monitoring process. Adults with a high caries risk profile were screened and randomly assigned to one of two study groups: treatment group (NaOCl and povidone-I2) and control group (povidone-I2). Two commercially available methods for assessing microbial populations were used on each adult. The methods are CRT® Bacteria (Ivoclar Vivadent Inc., Schaan, Liechtenstein; Amherst, NY, USA) and the CariScreen® test (Oral BioTech, Albany, OR, USA). Salivary samples were collected immediately before the first treatment, 1 week, 4 weeks, 8 weeks and 12 weeks .

After signing the Informed Consent Form, a visual examination of the oral cavity was made to verify the inclusion/exclusion criteria. Before the start of the study, a statistician used a table of random numbers to generate a list of TX and CT participant numbers. After the dental examination, the only person who knew which participant was in the treatment or control group gave the participant the appropriate rinse. See Figure 1. If the participant was in the treatment group, they gave a saliva sample for the CRT® test and then had the lingual surfaces of the lower anterior teeth swabbed with a chemically treated cotton

swab for the CariScreen® test. They would then rinse with 15 ml of the 1.6% solution of NaOCl for 60 seconds followed by rinsing with 15 ml of the povidone iodine for 60 seconds. They were offered a toothbrush and paste to offset the taste of the povidone iodine. At one week, they would have a CRT® test and CariScreen® test repeated. At one month, two months and three months they would repeat the CRT® test and CariScreen® test and each time follow with their appropriate rinses. If the participant was in the control group, they gave a saliva sample for the CRT® test and then had the lingual surfaces of the lower anterior teeth swabbed with a chemically treated cotton swab for the CariScreen® test. They would then rinse with 15 ml of the povidone iodine for 60 seconds. After rinsing, the participants were allowed to brush with toothpaste. The microbial monitoring was done at baseline, 1 week, 4 weeks, 8 weeks and 12 weeks. The 1.6% solution of NaOCl was prepared fresh daily using Clorox® and nonionized water. Betadine® (10% povidone-I2) was used for the povidone rinse (21). They were offered a toothbrush and paste to offset the taste of the povidone iodine. At one week, they would have a CRT® test and CariScreen® test repeated. At one month, two months and three months they would repeat the CRT® test and CariScreen® test and each time follow with their control rinse.

A meter reading <1500 is considered a low microbial load and a reading >1500 indicates a high microbial load [11,13]. The CariScreen® meter was calibrated before each sample was taken. For the CRT® Bacterial Test, the readings of <105 and >105 were made by comparisons with photographic standards that are specific for S. mutans and Lactobacillus [12]. After incubating, the readings were performed independently by two co-authors . Next, a DMFT index examination (baseline) was performed. The participants rinsed with 15 ml for one minute of either the randomly assigned treatment (NaOCl followed by the povidone-I2) or control mouth rinse (povidone-I2). Before the start of the study, a statistician used a table of random numbers to generate a list of Treatment and Control participant numbers. The dental examiner was blind to the assignment numbers.

This was followed by microbial monitoring in the following order: a saliva sample is taken for the CRT® Bacterial Test followed by the CariScreen® Caries Susceptibility Test. In the CariScreen® Caries Susceptibility Test, the lingual surfaces of the lower anterior teeth are swabbed firmly with a chemically treated cotton swab and placed in a meter.

Tooth Status. Tooth status (DMFT) was assessed using the NIDCR criteria [14] modified to the ICDAS (International Caries Detection and Assessment System) criteria [15] that were applied to coronal and root surfaces separately using the criteria in Table 1.3.

Statistical Analysis

Statistical analysis was based on group comparisons of the microbial levels between the treatment and control groups by each of the two methods for measuring microbial levels. Also, the tendencies in proportions of the microbial levels over different time points were tested among two groups separately. It was hypothesized that the treatment group (NaOCl and povidone-I2) would have significantly lower microbial counts than the control group and maintain lower microbial counts for up to 12 weeks.

Data were submitted to descriptive using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina, USA).

For dichotomized CRT® outcome ($<10^5$ and $>10^5$), the McNemar test aims to test the change in proportion between baseline and each follow-up time (1 week, 4 weeks, 8 weeks and 12 weeks). For continuous outcome variables like

measurement in CariScreen®, we applied a paired t-test to examine the change between baseline and follow-up times. Fisher's exact test was used to assess differences between the two study groups for categorical variables. Cochran's Q test is used to determine the difference in a dichotomous dependent variable over time. The Generalized Linear Mixed Model (GLMM) with the interaction between group and time helped to test the difference of readings over time between two groups. The Benjamini-Hochberg Procedure (BH) was used to adjust for multiple McNemar tests. It adjusts the rate to control false positive (i.e., reject the true null hypotheses incorrectly). All tests were two-sided and the p values of less than 0.05 were considered statistically significant. Multiple Imputations were performed by groups using package *mice* [18] in R Statistical analysis (Version 3.6.1) was based on group comparisons of the microbial levels between the treatment and control groups by each of the two methods for measuring microbial levels. Also, the tendencies in proportions of the microbial levels over different time points were tested among two groups separately.

For dichotomized CRT® outcome (<105 and >105), the McNemar test aims to test the change in proportion between baseline and each follow-up time (1 week, 4 weeks, 8 weeks and 12 weeks). For continuous outcome variables like measurement in CariScreen®, we applied a paired t-test to examine the change between baseline and follow-up times. Fisher's exact test was used to assess differences between the two study groups for categorical variables. Cochran's Q test is used to determine the difference in a dichotomous dependent variable over time. The Generalized Linear Mixed Model (GLMM) with the interaction between group and time helped to test the difference of readings over time between two groups to adjust for multiple McNemar tests. It adjusts the rate to control false positive (i.e., reject the true null hypotheses incorrectly). All tests were two-sided and the p values of less than 0.05 were considered statistically significant. Multiple Imputations were performed by groups using package mice15 in R (Version 3.6.1) [16].

After the dental examination, the only person who knew which participant was in the treatment or control group gave the participant the appropriate rinse. If the participant was in the treatment group, they would rinse 60 seconds with 15 ml of povidone-I2 followed by a 60 second rinse with 15 ml of the NaOCl. Before the microbial sampling, the participant was given a toothbrush and tube of toothpaste and allowed to brush. The participant then gave a saliva sample for the CRT® test and then had their lower anterior teeth swabbed with a chemically treated cotton swab for the CariScreen® test. The participant repeated the microbial sampling at one week, one month, two months and three months. If the participant was in the control group, they would rinse with 15 ml the povidone-I2. They would then proceed to tooth brushing and microbial sampling consisting of the CRT® test and CariScreen® test. They also gave samples at one week, one month, two months and three months. The sample size calculation is based on repeated measures comparing the microbial counts between the treatment and control groups. With a type I error of 0.05, type II error of 0.2 (or 80% power) and a two-sided test, a sample size of 10 in each group. This will allow an effect size of 1.3 in standard deviation units, which is statistically and clinically meaningful based on previous clinical studies. Assuming an attrition rate of 20%, the starting sample size needed is 25 adults [17].

Figure 1 summarizes which tests the Treatment and Control Groups received for each appointment.

Figure 1: Sequences of Treatments for Participants

Appointment	
Screening	Examine potential subjects for caries experience (DMFT) and interview for inclusion/exclusion
	Randomize subjects who meet inclusion criteria into two groups:
	Treatment Group / **Control Group**
Baseline	- The CRT® Bacteria Test - The CariScreen® Caries Susceptibility - Rinse with 15 ml of 1.6% solution of NaOCl for 60 seconds - Rinse with 15 ml of 10% povidone iodine for 60 seconds - The CRT® Bacteria Test - The CariScreen® Caries Susceptibility - Rinse with 15 ml of 10% povidone iodine for 60 seconds
One Week	- The CRT® Bacteria Test - The CariScreen® Caries Susceptibility - The CRT® Bacteria Test - The CariScreen® Caries Susceptibility
4 Weeks and 8 Weeks	- The CRT® Bacteria Test - The CariScreen® Caries Susceptibility - Rinse with 15 ml of 1.6% solution of NaOCl for 60 seconds - Rinse with 15 ml of 10% povidone iodine for 60 seconds - The CRT® Bacteria Test - The CariScreen® Caries Susceptibility - Rinse with 15 ml of 10% povidone iodine for 60 seconds
12 Weeks	- The CRT® Bacteria Test - The CariScreen® Caries Susceptibility - Rinse with 15 ml of 1.6% solution of NaOCl for 60 seconds - Rinse with 15 ml of 10% povidone iodine for 60 seconds - The CRT® Bacteria Test - The CariScreen® Caries Susceptibility - Rinse with 15 ml of 10% povidone iodine for 60 seconds

Figure 1: Appointments for Treatment and Control Groups

Results

There were 48 participants in this study (15 males and 33 females). There was no significant difference ($p<.05$) in the proportion of males between the Treatment and Control groups. The average age of the study group was 39.4 years (SD 8.6). The majority of the participants, 84% in the TX group and 86% in the CT group, were able to detect obvious gross tooth decay in their mouths. While all participants had to have at least two caries risk or disease factors, many of the participants did not receive fillings in the last three years (83% in the TX group and 84% in the CT group). 83% of the participants had snacked between meals or drank coffee, tea or soda with sugar with an average frequency of snacking 2.3 (0.98) for the TX group and 2.2 (0.92) for the CT group. There were no statistically significant differences between the TX and CT groups among all of the caries risk factors in the Caries Management by Risk Assessment (CAMBRA).

The frequency of brushing and flossing for the two study groups was almost identical with no significant difference detected. However, the frequency of flossing was slightly higher in the CT group (3.28 versus 4.83). 52% (TX group) and 35% (CT group) did not floss. There were no statistically significant differences for any brushing and flossing related questions.

The dental caries experience of the participants is presented in Table 1.A. There was no difference in any caries experience between two groups significantly at 0.05. The intensity of the decayed teeth in the TX group is more clearly reflected in the Sub-classification of Decayed Teeth in Table 1.B.

Table 2 **(CRT® Test)** presents the microbial changes that occurred over the 12 weeks of the trial for the TX and CT groups using the **CRT® Test** that is specific for *S. mutans* and Lactobacillus. The upper portion of the table shows the results for the *S. mutans* and the lower portion for the Lactobacillus results. Since there are missing data points at the various sampling times, the statistical technique of imputation preserves the data by replacing the missing data with estimates at each sampling point. The results of each statistical test were aggregated. Cochran's Q test was used to determine whether there was any difference in the dichotomous scoring of < 105 and > 105 microbial counts over the 12 weeks. The McNemar test compared the difference between baseline and each follow-up week. The results of Cochran's Q test showed that no significant changes occurred over the 12 weeks for either the TX group or CT group for both S. mutans and Lactobacillus measurements. However, starting at week 4 the number of colonies of S. mutans <105 TX was always greater than the number of colonies <105 for the CT group over the 12 weeks of the study.

Figures 2.A. and 2.B. presents an average proportion of microbial counts $>10^5$ for *S. mutans* and Lactobacillus by treatment and control group over 12-week follow-up visits. The TX group was able to keep the *S. mutans* members groups down for four weeks. The *S. mutans* count decreased from baseline to week 4 as low as 20% and then bumped up to 31% at week 12, while it generally increased for the control group from 33% to 52%. However, the Generalized Linear Mixed Model for the interaction of follow-up time and treatment group had a p-value of 0.06, which implies that no significant difference was detected on changes over time between treatment and control groups. Meanwhile, the counts of Lactobacillus went down simultaneously with no significant difference between the two groups (from 62% to 42% for the treatment group and from 63% to 38% for the control group) except for the first-week follow-up visit. The CariScreen® Meter Readings for both groups stayed flat for the first week of treatment and then increased largely to be double at week 12

Table 1.A. Caries Experience of Treatment and Control Groups

	Treatment Group Avg. (Std Dev)	Control Group Avg. (Std Dev)	Total Avg. (Std Dev)	p-value [a]
DMFT = Decayed, Missing, Filled Teeth	17.76 (5.59)	17.04 (4.01)	17.41 (4.86)	0.61
DT = Decayed Teeth	9.48 (4.98)	8.39 (3.76)	8.96 (4.43)	0.40
MT = Missing Teeth	4.28 (4.11)	5.00 (2.84)	4.63 (3.55)	0.49
FT = Filled Teeth	4.44 (4.35)	3.65 (4.46)	4.06 (4.37)	0.54
Number of teeth present	27.72 (4.11)	27.00 (2.84)	27.38 (3.55)	0.49
DMFT, %				
D	52.09%	49.23%	50.77%	0.84
M	23.52%	29.23%	26.15%	0.65
F	24.40%	21.54%	23.08%	0.81

[a] Two-sample T-test

Table 1.B. Sub-classification of Decayed Teeth for Treatment and Control Groups

		Treatment Group				Control Group				p-value [a]			
		D	D1	D2	Dx	D	D1	D2	Dx	D	D1	D2	Dx
Coronal Surfaces	Mean	4.20	3.16	0.68	1.16	4.91	1.69	0.65	0.82	0.36	0.06	0.90	0.43
	Std Dev	2.30	3.18	0.80	1.70	2.95	2.06	0.83	1.15				
Root Surfaces	Mean	0.56	0.44	0.00	N/A	0.43	0.04	0.00	0.00	0.70	0.15	---	---
	Std Dev	1.15	1.29	0.00	N/A	1.20	0.21	0.00	0.00				

[a] Two-sample T-test

D = White spot/enamel roughness detected with explorer

D1 = Clinically obvious decay

D2 = Only shell of crown exist

Dx = Only roots remaining

Table 1.C.: Criteria Used in Dental Caries Assessment

Study Criteria	ICDAS Criteria
SOUND (S) - Sound coronal surface/roots - Intact pit and fissure sealants - Non-vital teeth with sealed filling	
DECAY **D** = White spot/enamel roughness detected with explorer **D1** = Clinically obvious decay **D2** = Only shell of crown exist **DX (D3)** = Only roots remaining	1 and 2 3 and 4 5 and 6 ------
MISSING TEETH Missing teeth were classified into three categories. **M** = Tooth was lost as a result of caries or periodontal destruction; including clinically missing third molars. **ME** = Tooth was lost with space closed so that contact exists with adjacent teeth (probably lost for orthodontics reasons). **MT** = Tooth was lost due to trauma (verified by questioning of the subject).	NA
FILLED TEETH Defined as a tooth was a sound filling, permanent or temporary. Types of restorations and their acceptability were classified as follows: **F** = Sound filling, including inlay/ onlay. **CF** = Crown filling. **PF** = Pontic restoration. **DF** = Defective fillings were defined according to the "Victor" criteria of Ryge and Snyder[18] which included assessment of the surface and color (anterior teeth), anatomic form and marginal integrity. "Victor" is defined as clinical quality so unacceptable that it must be "replaced and/or immediately treated because damage is now occurring or because serious inadequacies exist".	NA

Table 2: Microbiological Changes Over 12-Week Period for Treatment and Control Groups for CRT® by Dichotomous Counts

		Baseline			1 Week			4 Weeks			8 Weeks			12 Weeks			p-value	p-value Baseline vs.			
		Strep Mutans																			
	N	n	$<10^5$	$>10^5$	n	$<10^5$	$>10^5$	n	$<10^5$	$>10^5$	n	$<10^5$	$>10^5$	n	$<10^5$	$>10^5$		1 wk.	4 wks.	8 wks.	12 wks.
Treatment	25	24	13	11	23 [b]	14	9	20 [c]	18	2	15 [d]	14	1	14 [e]	12	2	0.09	0.72	0.36	0.55	0.55
Control	23	21	14	7	20 [g]	14	6	19 [h]	14	5	12 [i]	11	1	13 [j]	8	5	0.26	0.72	0.44	0.56	0.56
Total	48	45	27	18	43	28	15	39	32	7	27	25	2	27	20	7	0.25	0.56	0.53	0.53	0.53
		Lactobacillus																			
	N	n	$<10^5$	$>10^5$	n	$<10^5$	$>10^5$	n	$<10^5$	$>10^5$	n	$<10^5$	$>10^5$	n	$<10^5$	$>10^5$		1 wk.	4 wks.	8 wks.	12 wks.
Treatment	25	24	9	15	23	9	15	20 [c]	10	9	15 [d]	7	8	14 [e]	9	5	0.11	0.79	0.43	0.43	0.36
Control	23	22	7	15	20 [g]	10	10	19 [h]	10	10	12 [i]	7	5	13 [j]	9	4	0.13	0.72	0.44	0.45	0.44
Total	48	46	16	30	43	19	25	39	20	19	27	14	13	27	18	9	0.04*	0.37	0.36	0.36	0.04*

* $p < 0.05$

[1] Cochran's Q test after Multiple Imputations; [2] McNemar test after Multiple Imputations. The average for the Treatment group was 40.4 years (SD 10.2) and Control group was 37.3 years (SD 6.6).

Treatment Group:

Strep Mutans

a Baseline: Patients #1 and 3 had no results
b 1 Week: Patient #29-no Strep results for 1 week
c 4 Weeks: Patients # 3, 19, 21, 23, 25 last week of results
d 8 Weeks: Patients # 3, 5, 7, 9, 13, 19, 21, 23, 25 no longer in study; #54 no 8-week data
e 12 Weeks: Patients #3, 5, 7, 9, 13, 19, 21, 23, 25 no longer in study and #44 and 52 no 12-week data

Lactobacillus:

a Baseline: Patients #1 and 3 had no n results
c 4 Weeks: Patients 3, 19, 21, 25 last week of results
d 8 Weeks: Patients #3, 5, 7, 9, 13, 19, 21, 23, 25 no longer in study #54 no 8-week data
e 12 Weeks: Patients #3, 5, 7 ,9, 13, 19, 21, 23, 25 no longer in study; #44 and 52 no 12-week data

Control Group:

Strep Mutans:

f Baseline: Patients #2 and 32 no results
g 1 Week: Patient #22 no longer in study; #30, 53 no 1 week data
h 4 Weeks: Patients #16, 20, 22, 26 no longer in study
i 8 Weeks: Patients #2, 4, 6, 8, 10, 16, 20, 22, 26, 41 no longer in study; #24 and 47 no 8-week data
j 12 Weeks: Patients #2, 4, 6, 8, 10, 16, 20, 22, 26, 41 no longer in study

Lactobacillus:

k Baseline: Patient #32 no results
g 1 Week: Patients 22 no longer in study; #30, 53 no 1-week data
h 4 Weeks: Patients 16, 20, 22 and 26 no longer in study
i 8 Weeks: Patients #2, 4, 6, 8, 10, 16, 20, 22, 26, 41 no longer in study; #24 and 47 have no 8-week data
j 12 Weeks: Patients #2, 4, 6, 8, 10, 16, 20, 22, 26, 41 no longer in study

Table 3: Microbiological Changes Over 12-Weeks for Treatment and Control Groups for CariScreen®: Dichotomous and Meter Readings

Group	Baseline			1 Week			4 Weeks			8 Weeks			12 Weeks			p-value [1]	p-value [2] Baseline vs:			
	n	<1500	>1500	n	<1500	>1500	n	<1500	>1500	n	<1500	>1500	n	<1500	>1500		1 wk.	4 wks.	8 wks.	12 wks.
TX	25	9	15	23 [b]	14	9	20 [c]	7	13	15 [d]	4	11	14 [e]	0	14	< 0.001**				
Mean		217.3	5,082.9		465.1	7,003.0		319.3	7,534.4		520.5	6,612.6		--	6,704.2		0.83	0.11	0.10	0.01*
Std Dev		187.9	2,986.6		364.3	2,619.2		232.3	1,819.2		394.7	2,394.0		--	2,026.1					
Control	23	12	10	23 [g]	13	10	19 [h]	8	11	10 [j]	0	19	13 [j]	1	12	< 0.001**				
Mean		461.8	5,808.9		485.4	5,414.9		449.9	6,579.1		--	7,511.0		111.0	6,431.5		0.17	< 0.00*	< 0.00*	< 0.00*
Std Dev		275.8	2,738.1		378.7	3,686.3		263.8	1,610.2		--	2,185.4		--	2,220.0					
Total	48	21	25	46	27	19	39	15	24	25	4	11	27	1	26	< 0.001**				
Mean		357.0	5,373.3		474.9	6,167.2		388.9	7,096.5		520.5	7,040.4		111.0	6,578.3		0.81	0.07	0.04	0.00*
Std Dev		267.1	2,854.2		364.2	3,241.4		249.9	1,757.9		394.7	2,286.1		--	2,079.0					

TX = Treatment; * p-value < 0.05; ** p-value < 0.001.

[1] Cochran's Q test after Multiple Imputations; [2] Paired T-test after Multiple Imputations.

Treatment Group:

a Patient #1 had no baseline reading result
b Patient #3 and #46 have no 1-week reading
c Patient #3, 19, 21, 23, 25 no longer in study after 1 week
d Patient #3,5,7,9,13,19,21,23, 25 no longer in study; #54 no 8-week reading
e Patient #3, 5, 7, 9, 13, 19, 21, 23, 25, 44, 52 no 12-week reading

Control Group:

f Patient #2-had no baseline reading result
g Patient #16, 20, 22, 26 no longer in study after 1 week
h Patient #2, 4, 6, 8, 10, 16, 20,22,26,41 no longer in study
i Patient #28, 47, 49 no 8-week result
j Patient #2,4,6,8,10,16,20,22,26,41 no 12-week reading

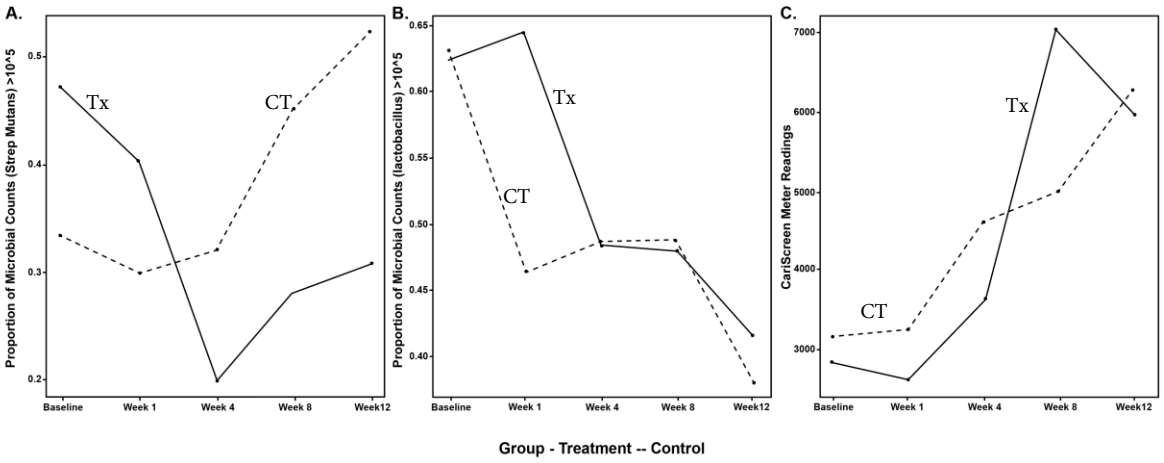

Figure 2: Average Microbial Counts for CRT® and Readings for CariScreen® by Treatment and Control Group Over 12-Week Follow-ups

Figure 2.A. Proportion of Strep Mutans Counts > 10^5

Figure 2.B. Proportion of Lactobacillus Counts > 10^5

Figure 2.C. Meter Readings in CariScreen®. Data after Multiple Imputations

of the values measured at the baseline, as is shown in Figure 2.C.

Table 3 (CariScreen® Test) presents the microbial changes that occurred over the 12 weeks of the trial for the TX and CT groups using the CariScreen® Test. The results are presented by dichotomous readings and meter readings. Using the dichotomous cutoff points of <1500 (low caries activity) and >1500 (high caries activity), Cochran's Q test suggested that significant changes occurred in both the TX and CT groups over the 12-week study.

The actual numerical meter readings of the CariScreen® test appear in the lower half of the table. The meter readings for both TX and CT groups separately and also when combined were all significant at 12 weeks. The p values for the t-test are based on comparisons with the baseline and each exam time. In general, the meter readings showed more fluctuations than the dichotomous scores. At one week, both the TX group and CT group were almost identical to the baseline readings indicating that both groups were able to keep the total microbial load low for one week. Figure 2.C more vividly shows that for both the TX and CT groups. The total microbial load stayed flat for one week before increasing unrelated to the CRT® test.

Discussion

This study did show that it is possible to keep the level of microorganisms relatively low for one week after a single rinse of either the TX rinses or CT rinse. In this study, the results of the CariScreen® Test, which measures the total number of all microorganisms present compared the CRT® Test, which specifically measures only the number of Streptococcus mutans present, were erratic after week one. If the CariScreen® Test would have paralleled the results of the CRT®, it would have been a useful chairside test of high bacterial levels in real time for the clinician. The cost of the test materials for the CRT® test is slightly more than the cost of the CariScreen® swabs. However, the cost of the CariScreen® meter is very expensive compared to the cost of a small incubator for the CRT® test.

The time trend test of the dichotomous scores over the 12 weeks was not significant for both the TX and CT groups. Both the TX and CT groups (CRT®) were effective in keeping the Lactobacillus low for 12 weeks. Also, the CT group had increasing S. mutans readings, while the readings dropped down over the 12-week period. The Null Hypothesis is that TX has the same effect as CT. Hence, the null hypothesis was both the TX and CT groups were effective in keeping the microbial levels down. In high caries risk adults, other organisms such as non-mutans bacteria and non-mutans streptococci [18] may have overshadowed the two organisms that were assessed [22].

An alternative to using povidone-I_2 as a rinse is to use a cotton roll saturated with povidone-I_2 to wipe the tooth surfaces. This would minimize the metallic taste factor without sacrificing the antimicrobial properties and decreasing the possibility of baceteremia.

Although the use of NaOCl proved to enhance the effect of Povidone-I_2, it was done to prove a point. Povidone-I_2 by itself can be used clinically for a variety of clinical conditions. It has been used to decrease gingivitis [6], to decease bacteremia in scaling [19], before gingivectomy in periodonfal surgery [20], injecting into periodontal pockets [7], to name a few clinical conditions. In general it could be used before any invasive dental procedure [21] .

What is needed in future antimicrobial studies is to try the use of the NaOCl at a weaker concentration (0.3%). NaOCl also acts by direct contact, but also has neutralizing properties and is more amenable as a mouth rinse than povidone-I2. Rather than follow a one-time rinse

schedule, a more frequent use similar to what is recommended for high caries risk adults in CAMBRA (i.e., once per day for one week a month) could be employed. The benefits of decreasing the total microbial load with NaOCl, low cost, no hypersensitivity issues and decreasing the possibility of bacteremia.

Study Limitations

Even though this was a pilot study the sample size was small was large enough to meet the criteria for significant differences. Also, the commercial methods used to sample the microbial levels are sensitive and problematic. Ideally, it would have been desirable to have a negative control (NaOCl only) instead of the povidone-I2 (positive CT). However, the positive control was used to determine if the NaOCl (TX) could enhance the effect of the povidone-I2povidone-I2. The sample was too small to include a third group. Although the povidone-I2 has a metallic taste that some might consider unpleasant, it did not prompt any of the participants to drop out of the study. The participants who did drop out of the study did so because they moved away, got a job, or missed their microbial sampling appointment by more than one day.

Conclusion

The use of NaOCl before the applying povidone-I_2 did enhance the antimicrobial properties of the povidone-I_2.

Products used in the methods section

Betadine® (10% povidone-I2) topical antiseptic bactericide (Avrio Health LP, Stamford, CT, USA).

CariScreen® (Oral BioTech, Albany, OR, USA).

Clorox® (The Clorox Company, Oakland, CA USA)

CRT® Bacteria (Ivoclar Vivadent Inc., Schaan, Liechtenstein; Amherst, NY, USA).

Sterile I-Pak® (Presterilized disposable mouth mirror and double-ended dental explorer.) (AD Surgical, Sunnyvale, CA, USA)

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