Original Article

An In Vitro Comparison of Antimicrobial Effect of Nanosil and ChlorhexidineMouthrinses

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Abstract

Background and Aim: Regarding chemical methods of plaque control, use mouthrinses are more frequent than other methods. The objective of this research is making a comparison between the antibacterial effect of Nanosil and chlorhexidine mouthwashes.

Materials and Methods: In this experimental study samples were taken from supragingival and subgingival plaques of 15 patients and transferred to aerobic and anaerobic liquid culture environments. The number of the bacteria in both aerobic and anaerobic liquid environments were determined by spectrophotometer. Then, the samples were transferred from the liquid culture environment to the considered solid culture environment as a mixture of nanosil and chlorhexidine mouthwashes and placebo and the numbers of the growth colonies in the solid culture environment were counted in each group and compared with each other by t test.

Results: In both aerobic and anaerobic conditions, the number of the growth colonies, was depicted in ascending order the groups of chlorhexidine, nanosil, and placebo. Concerning chlorhexidine, there was no significant difference between the growth colonies in the two aerobic and anaerobic environments indicating the absolute antibacterial effect of this mouthwash.

Nanosil mouthwash had a significant statistical superiority in comparison with the placebo in both aerobic and anaerobic environments. Nanosil had a significant effect on anaerobic environment, meanwhile, the placebo indicated a superior effect in anaerobic environment.

Conclusion: Nanosil mouthwash can be applied as an effective antibacterial substance especially in an anaerobic environment, though chlorhexidine as a standard mouthwash has still the strongest effect in this field.

Key Words: Dental plaque - Mouth wash – Nanosil - Chlorhexidine digluconate

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Introduction

Recurrent Aphthous Stomatitis (RAS) is the most common cause of recurrent oral ulcers that affects 20% of the general population. These lesions are restricted to the oral mucosa and mostly seen in mucosa of the cheek and lips. The first episode of RAS most often begins in the second decade of life. The main known etiologies of aphthous are heredity, hematologic, and immunological abnormalities [1]. It appears hematologic deficiencies particularly serum iron, folate, and vitamin B12 are the etiology of aphthous [2]. Other possible factors

include trauma, emotional and mental stress, anxiety, and food allergies [3].

Antioxidants can prevent and/or delay oxidative damage to target molecules. All molecules including lipids, proteins, nucleic acids, and carbohydrates are potentially exposed to oxidative agents [4].

Common antioxidants include vitamins A, E, and C and carotenoid compounds. Recent studies have shown that every antioxidant is uniquely useful for the immune system. Therefore, a high level of one antioxidant is not as effective as the average action of all antioxidants together [5]. In a study by Cimen et al., there was a reduction in CAT, GSH PX, and AOP levels in erythrocytes and reduction in AOP and increase in MDA levels in plasma in RAS patients compared to control group [6].

In Saral's study, vitamins A, E, and C in serum and saliva of patients with aphthous was significantly lower than those in healthy control group. This was the first comparative study on saliva and blood antioxidant levels in patients with oral ulcers that showed weakening of non-enzymatic antioxidants in these patients [7]. Karincaoglu et al. studied enzymatic antioxidant levels of Super Oxide Dismatase (SOD), Catalase (CAT), and Glitathion peroxidase (GSHPX) in plasma and saliva, and uric acid in saliva of RAS patients. They found lower levels of CAT and SOD in plasma and a higher level of GSHPX in RAS patients compared to control group [8].

The nanosil mouthwash (Sanosil) (Kimiafam pharmaceutical Co., Iran) is a hydrogen peroxide formulation that contains few silver ions [6,7]. Hydrogen peroxide prevents proliferation of effective anaerobic bacterial mass in periodontal disease due to antimicrobial properties and oxygen release. The released oxygen destroys bacterial and viral protective membranes and renders nanosil capable of penetration, a mechanism through which microorganisms are destroyed [8].

Silver ions are incorporated as silver nanoparticles in nanosil mouthwash. Smaller particle sizes increased antibacterial properties to more than 99%. [6,7] Antibacterial effect of the silver ion is dependent upon extremely firm covalent bonds to bacterial proteins that leads to precipitation of proteins thereby inactivation of the bacteria. Both hydrogen peroxide and silver ions have synergistic effects. Manufacturer of the nanosil claims that it does not have any environmental harmful effects, because its main component, hydrogen peroxide, degrades to form water and oxygen which are not considered as polluting agents [8].

The aim of this study was to compare antibacterial effects of nanosil and chlorhexidine in both aerobic and anaerobic environments.

Materials and Methods

This experimental laboratory study involved 15 patients whose plaque samples were transferred to a laboratory. Patients with moderate to severe periodontitis were included and exclusion criteria comprised of samples with difficulty in sampling, for example lack of isolation, blood contamination of the samples or inability to approximate the sample with a flame while taking subgingival samples in an anaerobic environment. Sites with acceptable conditions for sampling were selected. Aerobic sampling was performed in any area having supragingival plaque and anaerobic sampling was carried out in teeth with pocket depths of equal to or more than 4 mm subgingivally. Two aerobic and anaerobic samples were taken from each patient. After case selection and before sampling the area was rinsed with copious amount of water to remove saliva and food debris. Irolation was done with placement of cotton rolls. Supragingival samples were taken using a curette. Then, aerobic samples were transferred to trypticase soy agar culture tubes. Subgingival samples were taken as stated previously without blood contamination and approximated with a fire flame while transferring to sodium tioglycolate culture medium. In case of blood contamination while sampling, another site was selected from which a new sample was taken. Culture media were incubated at 37 degrees Celsius for 24 hours. Samples were exposed to different mouthwashes according to the experimental groups they belonged to as follows:

Group 1: 0.2 chlorhexidine (Behsa pharmaceutical Co, Iran)
Group 2: Nanosil
Group 3: Normal Saline as placebo
After transferring the samples to the culture media, number of colony forming units were counted separately for each group. Different dilutions were made for culture media to render the colony formig units countable. Before application of the mouthwashes, the number of bacteria in both aerobic and anaerobic samples were counted and equalized for both groups using turbidity evaluation by spectrophotometry [13].
One milliliter of all dilutions of thioglycolate broth and trypticase soy broth were transferred to tubes containing 10 mL of each mouthwash and homogenized for 30 seconds. Then 0.1 mL of each tube was transferred to a solid blood agar culture. Culture plates containing anaerobic bacteria were transferred to a candle jar. The candle jar as well as the aerobic cultures wereincubated for 24 to 48 hours at 37 degrees Celsius. After 48 hours in all dilutions, appropriate dilution for each mouthwash was determined according to the number of colony forming units. Therefore, in both aerobic and anaerobic cultures ½ to ¼ dilution for nanosil, 1/3 to 1/5 for normal saline and 0 to ½ for chlorhexidine was determined. Eventually, 1 mL of each dilution was transferred to the tubes containing mouthwashes under sterile conditions. After homogenization for 30 seconds, 0.1 mL was added to the blood agar plates and diffused. Blood agar culture plates were placed in the candle jar for anaerobic cultur ing and were incubated accompanied with those used for aerobic culturing at 37 degrees celcius for 48 hours. The number of colony forming units were counted for each plate. Then the mean for each colony in different dilutions for each mouthwash was calculated in both aerobic and anaerobic conditions. Independent t-test was used to compare the mean colony forming units in both aerobic and anaerobic cultures.

Results

The number of colony forming units while being subjected to chlorhexidine was zero in almost all appropriate dilutions. Since the 10^{-4} dilution was common in nanosil and normal saline, for counting the number of colony forming units, this dilution was used in order to compare the effect of nanosil and normal saline and chorhexidine was disregarded. There was a significant difference between the number of colony forming units of nanosil and placebo under both aerobic and anaerobic conditions, according to the t-test ($p<0.001$). Also, there was a significant difference between the number of colony forming units when the samples were subjected to nanosil under aerobic and anaerobic conditions ($p>0.004$). There was also a significant difference between aerobic and anaerobic culture conditions at the presence of placebo ($p<0.001$). Comparison of the number of colony forming units in different mouthwashes is depicted in table 1.

Table 1: Comparison of the number of colony forming units in different experimental groups

	Aerobic culture			Anaerobic culture		
	Standard deviation ±mean	**Maximum**	**Minimum**	**Standard deviation ± mean**	**Maximum**	**Minimum**
Nanosil	23/6± 6/9	150	10	49/6±34/7	39	12
Placebo	223/5±70/6	1925	300	891/5±530/6	352	102

Discussion

The aim of this study was to evaluate and compare antibacterial effects of nanosil and chlorhexidine. Studies about CHLORHEXIDINE have shown its high antibacterial influence with respect to other similar agents. Few antibacterial mouthwashes have been stated to be comparable with CHLORHEXIDINE in terms of antimicrobial properties [14,15]. Scarse studies can also be found to reveal superiority of other antimicrobial agents in comparison with CHLORHEXIDINE [16]. Superior antibacterial effect of CHLORHEXIDINE in comparison with oxidizing agents have also been depicted by Moran [11] Gusberti [12] and Menendez [14]. Kazemi and co-workers stated that CHLORHEXIDINE had a stronger effect on reducing plaque and gingival indices in comparison with nanosil, but there no significant difference between them in decreasing bleeding index. Tooth discoloration was significantly less in nanosil than in CHLORHEXIDINE. Our results indicated that CHLORHEXIDINE hadstronger antibacterial influences than nanosil in both aerobic and anaerobic culture conditions. Since antibacterial effect of each group is assessed separately and meanwhile comparison is not logical and the number of colony forming units was zero for CHLORHEXIDINE, the CHLORHEXIDINE group was disregarded in statistical analyses and comparisons of the colony forming units were carried out in nanosil and placebo groups, using t-test. It was demonstrated that nanosil had stronger antibacterial effects specifically in anaerobic conditions. This can be attributed to the release of oxygen from peroxide groups incorporated in nanosil. Decreasing the number of colony forming units in placebo group can show that addition of an isotonic solution to the culture medium can decrease growth of microorganisms. It could not be clearly justified why the decrease in number of colony forming units was more in anaerobic cultures than in aerobic ones at the presence of placebo.

Therefore the null hypothesis of antimicrobial effect of these two mouthwashes and the difference between these two agents in their bacterial growth control is accepted.

Conclusion

Although chlorhexidine has a strong antimicrobial effect, it could be concluded that nanosilwas also able to act as an effective antibacterial agent especially in anaerobic conditions.

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