COMBINED SEA SALT-BASED ORAL RINSE WITH XYLITOL IN ORTHODONTIC PATIENTS: CLINICAL AND MICROBIOLOGICAL STUDY

A. BALLINI[1*], S. CANTORE[1,2*], E.A. FOTOPOULOU[3*], I.P. GEORGAKOPOULOS[3], E. ATHANASIOU[3], D. BELLOS[3], G. PADUANELLI[4], R. SAINI[4], G. DIPALMA[4*] and F. INCHINGOLO[4*]

[1]Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari Aldo Moro, Bari, Italy, [2]POLYPHENO S.r.l., Accredited Spin Off, University of Bari Aldo Moro, Bari, Italy; [3]Dentist Education Institute, Athens, Greece; [4]Department of Interdisciplinary Medicine, School of Medicine, University of Bari Aldo Moro, Bari, Italy

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*These Authors contributed equally to this work

Orthodontic appliances protect the plaque from the actions of brushing, mastication, and salivary flow. Plaque control is a very difficult task for patients with fixed orthodontic appliances. It has been demonstrated that chemical agents are useful additions in plaque control for these patients. To improve mechanical plaque removal, studies in the field suggest to add a chemo–therapeutic agent, such as an antibacterial mouth rinse, to the oral hygiene regimen. Noteworthy evidence from clinical trials has shown that oral hygiene status is significantly improved when antibacterial mouth rinses are added to daily oral hygiene measures (tooth brushing and flossing) compared with tooth brushing and flossing alone. The purpose of this study was to evaluate the effect of a combined rinsing solution (sea salt, xylitol and lysozyme) on plaque and gingival index in orthodontic patients with fixed appliances.

To the Editor,

Dental plaque, which is typically accumulated during orthodontic treatment, is the major etiologic factor in the development of dental caries and gingivitis (1). Orthodontic metallic attachments may cause alterations in the oral microflora due to variation of pH and bacteria affinity to the metallic surfaces facilitated by electrochemical reactions, that lead to the development of new plaque retentive areas, which, in turn, result in increased microbe carriage (2).

Generally, orthodontic patients are unable to maintain adequate oral hygiene by mechanical means alone due to the failure of plaque removal from difficult-to-access areas that are hindered by orthodontic attachments (3). A common strategy is to add a chemotherapeutic agent, such as an antimicrobial mouth rinse, to their mechanical oral hygiene routine (4).

Recent caries research has been mostly focused on xylitol, which is a caloric sugar substitute (5, 6). Most plaque bacteria lack the ability to ferment xylitol into cariogenic end products. Instead, xylitol accumulates intracellularly in *S. mutans* as a non-metabolizable metabolite, thus inhibiting bacterial growth, reducing bacterial population, and reducing

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Corresponding Author:
Dr. Stefania Cantore,
University of Bari Aldo Moro,
P.zza Giulio Cesare, 11,
70124, Bari, Italy
Tel./fax: +39 0805448514.
e-mail: stefaniacantore@pec.omceo.bari.it

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the amount of plaque. Moreover, the presence of sea salt in the mouth increases the pH level inside the mouth.

The goal of the present study was to investigate the impact of a unique mouth rinse formula (H2Ocean Sea Salt Mouthwash) on gingival health in orthodontic patients based on the reported health benefits from sea salt and xylitol in the oral cavity.

MATERIALS AND METHODS

Subjects and study schedule

Twenty orthodontic patients were enrolled in the study. During the study period the subjects underwent fixed orthodontic treatment in a private practice. The study was conducted as a double-blind parallel-group clinical trial. Written informed consent was obtained from patients in accordance with the Declaration of Helsinki.

The participants were asked to rinse their mouth twice a day for 4 weeks after brushing in the morning and at night before sleeping, and once per day for the following 4 weeks (before sleeping). The patients were instructed to rinse with 15 mL of the solution for 1 min followed by expectoration of the residual mouth rinse and avoid drinking and eating for 30 min after rinsing. To avoid the introduction of new variables, participants were asked to continue their usual daily brushing method during the study period.

Written instructions were given to the participants, explaining how to use the mouthwash. Rinsing was performed at home without supervision. To check for compliance, the participants were asked to note the times of day when they rinsed.

Sample size and blinding

All selected participants had mild gingivitis at the start of the study. All participants were randomly assigned to one of two treatment groups (n=10 in each group) by using two series of random numbers, one for the male participants and one for the female participants. The groups and assigned mouthrinses were as follows:

A: A combined mouth rinse containing sea salt,

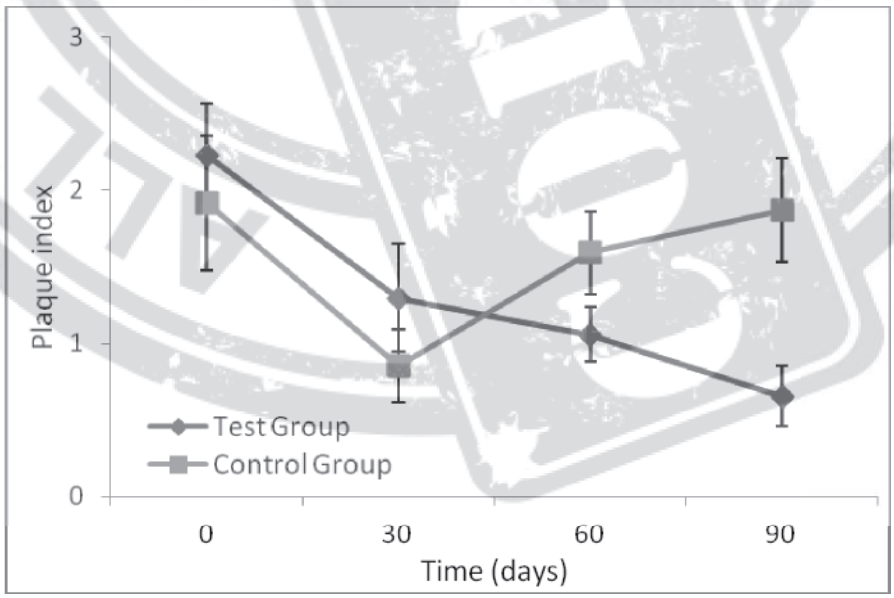

Fig. 1. *Effect of oral solution on the plaque index at baseline (day zero) and 30, 60, 90 days after tooth scaling and correct oral hygiene instructions. Control group received placebo. The index for each patient was obtained by summing the indices for all the examined teeth and dividing by the total number of the examined teeth. Results are presented as the mean±SD and significant differences between the test and control groups are indicated by * ($p<0.05$) or *** ($p<0.001$).*

lysozyme and xylitol (H2Ocean Sea Salt Mouthrinse, a new mouthwash formula that contains sea salt, xylitol and lysozyme with no harsh chemicals, alcohol or fluoride).

B: A placebo mouth rinse (physiologic saline solution - no mouthwash dilution added).

All of the mouth rinse bottles had a similar appearance.

Inclusion criteria

Patients aged between 18 and 30 years, and suffering from mild gingivitis, under orthodontic treatment, with good-to-fair oral hygiene were selected for the study.

Eexclusion criteria

Patients on antibiotic therapy or those who had been on antibiotic therapy in the previous month or during the trial period, suffering from pre-existing oral irritations or oral infections (rampant caries, severe/aggressive gingivitis and periodontitis, oral thrush, diagnosed halitosis, or diagnosed xerostomia), using any mouthwash within the previous month, smoking or consuming tobacco in any form, suffering from any systemic diseases, and patients who were deemed to be uncooperative.

Clinical parameters

For the clinical parameters, scores including Bleeding Index (BI) and Plaque Index (PI), (7-9) were blindly taken from all participants by the same trained examiner at baseline and after 30, 60, and 90 days (investigation of supplementary 30 days from the end of oral rinsing to assess the long-term efficacy of the product). The measurements were recorded from central incisors, canines and second premolars of four quadrants.

A mean value of each parameter for both groups, was calculated in each pre- and post-rinse measurement.

Microbiologic examination

For microbial evaluation, saliva samples were collected by chewing on a piece of sterile paraffin wax. We conducted paired cultivation on 20 samples in order to test whether there were variations in the bacterial population caused by the laboratory methods. The samples were transferred, immediately upon collection,

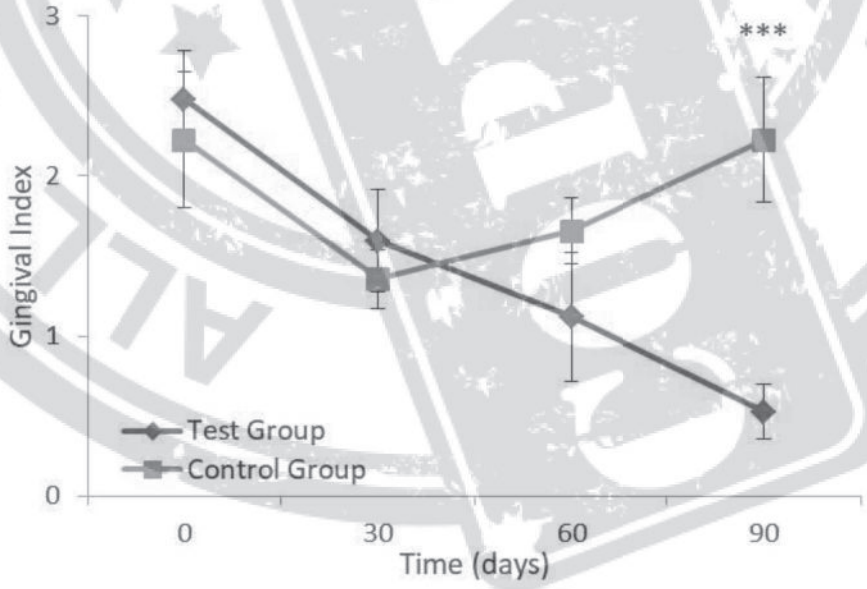

Fig. 2. *Effect of oral solution on the gingival index at baseline (day zero) and 30, 60, 90 days after tooth scaling and correct oral hygiene instructions. Control group received placebo. The index for each patient was obtained by summing the indices for all the examined teeth and dividing by the total number of the examined teeth. Results are presented as the mean±SD and significant differences between test and control group are indicated by * ($p<0.05$) or *** ($p<0.001$).*

to the Microbiology Laboratory for further processing. Then, 50 µL of the specimen were cultured in Blood Agar and Eosin Methylene Blue (EMB) mediums. The colony counts were enumerated in 48-h incubation at 37°C. The total bacterial count was determined by visual counting and was multiplied by 20 to express the count in terms of colony forming units (CFU)/mL (4).

Statistical analysis

Statistical analysis was carried out by utilizing Two-way ANOVA and Tukey's multiple comparison tests to assess the statistical differences. For this purpose, GraphPad Prism software (version 7.0; San Diego, California, USA) was used. The probability (P) of <0.05 was considered to indicate statistical significance.

RESULTS

Clinical measurements

We obtained encouraging clinical results in our study. The participants provided positive feedback on the use of our suggested hygienic protocol.

Plaque index

Fig. 1 shows the effect of the oral solutions on the plaque index at baseline (day zero) and 30, 60, 90 days after tooth scaling and oral hygiene home care instructions. The control group received placebo. The index for each participant was obtained by summing up the indices for all the examined teeth and dividing by the total number of the examined teeth for that participant. The results are presented as the mean ±SD of ten participants per group and significant differences between the test and the control groups are indicated by * ($p<0.05$) or *** ($p<0.001$).

Gingival index

Fig. 2 shows the effect of the oral solution on the gingival index at baseline (day zero) and 30, 60, 90 days after tooth scaling and oral hygiene home care instructions. The control group received placebo. The index for each participant was obtained by summing up the indices for all the examined teeth and dividing by the total number of the examined teeth for that participant. The results are presented as the mean ±SD of ten participants per group and significant differences between the test and the control groups are indicated by * ($p<0.05$) or *** ($p<0.001$).

Microbiologic examination.

Large variations in inter- and intra-individual measurements were observed. Both examinations

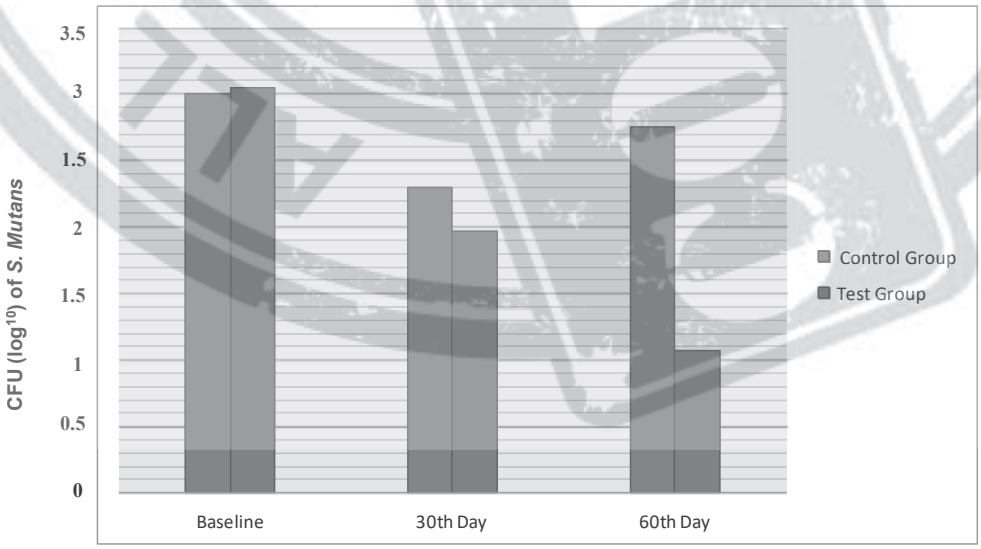

Fig. 3. *Intergroup comparisons of mouth rinses based on microbiological parameters.*

showed a significant decrease from the baseline.

Compliance.

Compliance with the course of the use of the tested product was documented. All participants completed the course of mouthwash protocol as indicated and returned the medication bottles.

DISCUSSION

The results of our study show a significant improvement of oral hygiene status when the natural antimicrobial mouth rinses were added to the daily oral hygiene regimen. The importance of such agent was even greater for the participants with oral appliances (7-11).

The present study was therefore designed to evaluate clinical and microbiological effects of H2Ocean Sea Salt Mouthwash when added to the oral hygiene regimen of orthodontic patients. We conducted a double-blind procedure in order to obtain unbiased results. The study groups were comprised of teenager and young adult orthodontic patients. Taking into consideration that such patients had difficulty in mechanical plaque control, it seems that they were an interesting category for this kind of study in terms of compliance. In order to better observe the efficacy of mouth rinses, all selected participants had mild gingivitis at the start of study.

One may try to attribute the observed findings to the fact that the participants knew about the goal of the study or to the mechanical effect of rinsing alone. Given that there was no improvement in the scores of the placebo group, the active mouth rinse effects could not be attributed to the Hawthorne effect or to the mechanical effect of rinsing alone (12). Subsequently, the limited number of published studies on the subject makes the comparison of our results with other groups rather limited. However, this research was a relatively short clinical study, and a longer and more detailed study may show a different outcome.

Finally, the clinical and *in vitro* data obtained in this study revealed that H2Ocean Sea Salt Mouthwash led to good inhibition of *S. mutans* and improved clinical conditions compared to those at baseline. We believe that a toothpaste or a mouthwash containing sea salt extract and xylitol, in combination with lysozyme, might be useful for the prevention of dental-related diseases.

REFERENCES

1. Tufekci E, Casagrande ZA, Lindauer SJ, Fowler CE, Williams KT. Effectiveness of an essential oil mouthrinse in improving oral health in orthodontic patients. Angle Orthod 2008; 78:294-98.
2. Ballini A, Santacroce L, Cantore S, Saini R, Mastrangelo F, Desiate A, Scacco S. Orthodontic forces modulate insulin-like growth factor binding protein-5 changes in gingival crevicular fluid. J Biol Regul Homeost Agents 2016; 30:1235-40.
3. Brightman LJ, Terezhalmy GT, Greenwell H, Jacobs M, Enlow DH. The effects of a 0.12% chlorhexidine gluconate mouthrinse on orthodontic patients aged 11 through 17 with established gingivitis. Am J Orthod Dentofacial Orthop 1991; 100:324-29.
4. Cantore S, Ballini A, Mori G, et al. Anti-plaque and antimicrobial efficiency of different oral rinse in a 3-days plaque accumulation model. J Biol Regul Homeost Agents 2016; 30:1173-1178.
5. Masoud MI, Allarakia R, Alamoudi NM, Nalliah R, Allareddy V. Long-term clinical and bacterial effects of xylitol on patients with fixed orthodontic appliances. Prog Orthod 2015; 16:35.
6. Cantore S, Ballini A, Farronato D, et al. Evaluation of an oral appliance in patients with mild to moderate obstructive sleep apnea syndrome intolerant to continuous positive airway pressure use: Preliminary results. Int J Immunopathol Pharmacol 2016; 29:267-73.
7. Ballini A, Scattarella A, Crincoli V, et al. Surgical treatment of gingival overgrowth with 10 years of follow-up. Head Face Med 2010; 6:19.
8. Ballini A, Tetè S, Scattarella A, et al. The role of anti-cyclic citrullinated peptide antibody in periodontal disease. Int J Immunopathol Pharmacol 2010; 23:677-81.
9. Mori G, Brunetti G, Colucci S, et al. Osteoblast apoptosis in periodontal disease: role of TNF-related apoptosis-inducing ligand. Int J Immunopathol Pharmacol 2009; 22:95-103.
10. Tatullo M, Simone GM, Tarullo F, et al. Antioxidant and antitumor activity of a bioactive polyphenolic fraction isolated from the brewing process. Sci Rep

2016; 6:36042.
11. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Sammartino G, Charrier JB. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in human cell cultures: growth factor release and contradictory results. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010; 110(4):418-21; author reply 421-22.
12. Campbell JP, Maxey VA, Watson WA. Hawthorne effect: implications for prehospital research. Ann Emerg Med 1995; 26:590-94.