In Vivo Evaluation of Antibacterial Toothpaste Efficiency and Patients' Satisfaction: a Double-blind Randomised Controlled Trial

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Study Protocol

This was a single-site, double-blind RCT that was conducted in the Dental Clinic of Bologna University (DIBINEM), Bologna, Italy in the period from November 2022 until July 2023. Before initiating the trial, the study protocol was reviewed and approved by the University Ethics Committee (793/2022/SPER/AUSLBO). The trial was in accordance with Helsinki Declaration of Human Rights [21] and followed the CONSORT statement. The tested dentifrices included a newly introduced ZCT-, HAP- and KCit- 1450 ppm sodium monofluorophosphate containing (Experimental group; Mentadent PROTECT+ Carie, Mentadent, Unilever, Milan, Italy) and 1450 ppm NaF containing (Control group; AZ Multi-Protezione Scudo Protettivo Famiglia) toothpastes.

Sample size, recruitment and eligibility criteria

The number of patients was determined based on the power analysis performed using data from a previously published study in order to assure a power of 80% for finding the statistically significant differences given the standard value of type I errors (0.05). Based on the outcomes of the power calculation (effect size f=0.2291667, at the level of α 0.05, and 1-β prob 0.80), the determined sample size was 92 patients. Considering the probability of loss to follow-up 10% of the minimum size per group was added, leading to the final sample size of 100 patients.

Potential patients were recruited through local advertisement or on-site, during routine dental check-ups. Study details were explained to the potential participants and only those who voluntarily signed the informed consent were scheduled for the first visit. During the screening phase, one dentist evaluated the eligibility of the patients in the study based on the following inclusion criteria: male and female patients aged 18-50 with minimum of 20 natural teeth in stable occlusion and good oral hygiene level (bleeding on probing not exceeding 20%, no advanced periodontal disease, absence of active caries lesions, pulpitis), patients with good general health (no systemic diseases reported in the medical anamnesis), and subjects with good language comprehension. Exclusion criteria were tooth anomalies (i.e. amelogenesis imperfecta, dentinogenesis imperfecta etc.), intrinsic stain (i.e. fluorosis, molar incisors hypomineralization [MIH]), active caries lesions, advanced periodontal disease, smokers, presence of orthodontic devices, use of antibiotics in the last 3 months, use of antibacterial mouthrinses in the last 3 months, reported allergies, drug and alcohol addiction.

Study procedures

One week before the beginning of the trial, all patients received sub- and supra-gingival full-mouth prophylaxis. At the same appointment, patients were given detailed oral hygiene instructions by the dental hygienist and a brief explanatory video was shared via

messaging applications (i.e., WhatsApp) to serve as a reminder for good habits consulting during the study period. The patients were scheduled to come back after 7 days (baseline visit) and were asked not to brush or eat at least 5 hours before the appointment.
During the baseline visit, all patients completed a custom-made questionnaire (supplementary file) containing questions regarding dental sensitivity ("self-reported" sensitivity), their perception of tooth color and overall perception of the toothpaste they were using at the time. Subsequently, they were asked to collect 2 ml of unstimulated saliva in sterile tubes (Greiner centrifuge tube, Sigma-Aldrich, St Louis, MO, USA). Saliva was immediately processed for microbiological analysis as thereafter described. Upon saliva collection, a sensitivity test was conducted by applying a tactile and air-stimulus (duration: 2 s, distance from the tooth surface: 1 cm) test to all teeth of the four quadrants. The subjects' response was recorded on a 4-point Schiff Sensitivity Scale [25] and visual-analogue scale, respectively. Additionally, plaque index (Modified Quigley and Hein) was scored on a 6 point scale at 3 buccal and 3 lingual sites per tooth after it had been disclosed with a disclosing agent (Biofilm Disclosure, EMS Dental, Nyon, Switzerland); gingival index according to Löe-Silness was also recorded, as well as bleeding on probing index. Patients' tooth color was determined under daily light using the Vita classical shade guide. Finally, at the end of the baseline visit, the participants were provided with the same soft-bristled toothbrush (Mentadent P, Mentadent, Unilever) and randomly assigned into one of the two groups (n=50) according to the dentifrice used. An online software (https://www.sealedenvelope.com) was utilized for randomization purposes by a staff member unaware of the study protocol. In order to enable complete blinding of the participants and dental examiner to the group assignment, the two dentifrices were distributed in identical plain white tubes provided with only letters A or B, with no other visible marks. The patient's code and dentifrice assigned (A or B) were documented in the chart for later reference. From the baseline visit, the subjects had to restrain from using any other products or means of oral hygiene except those provided and were invited to follow the domiciliary oral hygiene instructions as previously described. In case of any adverse events were to occur, the subjects were advised to discontinue the use of the toothpaste and were withdrawn from the study. The 4 weeks follow-up visit was fixed (recall).
After 4 weeks (recall), the patients returned to the study site for a final visit after having abstained from brushing or eating at least 5 hours before the visit. During this visit, the patients were asked to fill in the questionnaire (supplementary file), while the saliva collection and all clinical procedures described above were repeated by the same dentist.

Microbiological procedures
The saliva collected during the baseline and recall visits was processed for microbiological analysis within 3 hours of sample acquisition. Briefly, 0.1 ml of saliva was diluted in 9.9 ml of sterile phosphate-buffered saline solution until 10^{-8} was reached. Subsequently, the diluted samples were dispersed on Mitis Salivarius Selective Agar (MSB, Microbiol Diagnostici, UTA, CA, Italy) and the plates were incubated at 37°C for the next 48-72h. The bacterial colonies were identified by morphology and counted by one trained investigator, blinded to the groups. S. mutans colonies (CFU) were multiplied by their respective dilution ratio to calculate the number of colonies per one milliliter

(CFU/mL) of each subject's saliva sample. In order to perform statistical analysis, a base-10 logarithm transformation was applied to the calculated CFU/ml values.

Statistical analysis

After checking the normality (Kolmogorov–Smirnov) and the homoscedasticity (modified Levene's test) of the data, the Two Way Repeated Measures ANOVA (One Factor Repetition) followed by Bonferroni post-hoc were run to investigate the effect of the tested dentifrices on salivary counts of S. mutans, as well as plaque and gingival indexes. Since the data retrived from questionnares where not normally distributed (Kolmogorov–Smirnov, $p<0.05$), the non-parametric Mann-Whitney U-test was run. All analyses were performed by a statistician blinded to the groups using SigmaPlot 14.0 (Systat Software, Chicago, IL, USA). In all tests, the significance level was set at $\alpha=0.05$.