DR. SANTOSH TADAKAMADLA (Orcid ID : 0000-0003-2775-2897)

PROF. CHIARELLA SFORZA (Orcid ID : 0000-0001-6532-6464)

DR. GIANLUCA MARTINO TARTAGLIA (Orcid ID : 0000-0001-7062-5143)

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Clinical efficacy of a new Cetyl Pyridinium Chloride-Hyaluronic Acid based mouthrinse compared to Chlorhexidine and placebo mouthrinses – A 21 days randomised clinical trial.

Santosh Kumar Tadakamadla,[1] Venkata V. Bharathwaj,[2] Prabu Duraiswamy,[2] Chiarella Sforza,*[3] and Gianluca M. Tartaglia*[4].

[1]School of Dentistry and Oral Health & Menzies Health Institute Queensland, Griffith University, Australia.

[2]Department of Public health dentistry, SRM Dental College, Bharathi Salai, Ramapuram, Chennai, Tamil Nadu, India.

[3]Department of Biomedical Sciences for Health, Functional Anatomy Research Center (FARC), Università degli Studi di Milano, Milano, Italy; Institute of Bioimaging and Molecular Physiology, National Research Council, Segrate, Italy; ORCID 0000-0001-6532-6464.

[4]Department of Biomedical Sciences for Health, Functional Anatomy Research Center (FARC), Università degli Studi di Milano, Milano, Italy; Private Practice, SST Dental Clinic, Segrate, Italy; ORCID 0000-0001-7062-5143.

* Drs Sforza and Tartaglia equally contribute to this investigation.

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CONSORT guidelines: This clinical trial is reported accordingly; the relevant checklist is enclosed

Corresponding author

Dr. Gianluca M. Tartaglia
Department of Biomedical Sciences for Health, Università degli Studi di Milano,
Via Luigi Mangiagalli 31 - 20133 Milano, Italy
Phone +39 02 269 21196
Fax +39 02 503 15387
Email: gmtartaglia@gmail.com ; farc@unimi.it

Abstract

Objective: To compare the effectiveness of a novel Cetylpyridinium Chloride (CPC)-Hyaluronic Acid (HA) based mouthrinse with Chlorhexidine (CHX) and placebo mouthrinses in preventing plaque and gingivitis. The secondary outcomes were calculus, extrinsic stains, oral malodor and occurrence of adverse events.

Methods: 21-day randomised, double-blind, three-arm parallel study with random allocation of young dental students to any of the three mouthrinse groups. Thorough prophylaxis was done at baseline followed by a baseline examination for oral malodor, extrinsic stains, calculus, gingivitis, and plaque by a single examiner. All the subjects used the allocated mouthrinse twice daily for 21 days and were examined again at the end of the experimental period. They were also interviewed for adverse events. Change in the scores of clinical indices was calculated and compared between the groups.

Results: 75 subjects were included and completed the experiment. There was a significant difference for change in plaque index scores between the groups (p=0.015); subjects in placebo group experienced higher levels of plaque accumulation than the other groups. Teeth

staining increased in CHX ($p<0.001$) and placebo groups ($p=0.002$), but not in CPC-HA users ($p=0.573$). No significant differences were found between the three experimental groups for change in the gingival index ($p=0.08$), calculus scores ($p=0.494$), oral malodor ($p=0.870$) and reporting of adverse events ($p=0.249$).

Conclusions: CPC-HA and CHX had similar effectiveness in preventing plaque accumulation while no differences were observed between the mouthrinses for preventing gingivitis. Dental staining was caused by CHX and the placebo mouthrinses but not by CPC-HA mouthrinse.

Introduction

Microbial plaque is an etiological agent for gingivitis and controlling plaque plays an important role in the maintenance of good oral health[1]. Mechanical means of plaque control, e.g. tooth brushing, flossing and use of other mechanical devices are the most commonly used methods[2]. However, ideal plaque control solely by mechanical means requires a significant effort and is difficult to achieve. This has led to the introduction of chemical methods of plaque control using antimicrobial agents[3]. Efficacy of chemical agents in plaque control and preventing gingivitis is well documented[4]. They also help preventing oral malodor due to poor oral hygiene[5].

Currently, Chlorhexidine (CHX) mouthwash is widely used as a reference standard in research trials when testing the efficacy of various antimicrobial mouthrinses[6]. Although a reference standard, CHX had been associated with extrinsic staining, and increased calculus formation[7]. Due to these inadvertent effects of CHX, mouthrinses with Essential oils and Cetyl Pyridinium Chloride (CPC) have gained importance, they have been proved to be successful in preventing gingivitis and plaque[8].

There has also been an increasing demand for mouthrinses containing natural compounds both among the professional community and patients due to their plaque and gingivitis inhibiting effectiveness with minimal adverse events[8]. A recent systematic review observed that some mouthrinses with natural compounds as ingredients demonstrated significant benefits in preventing plaque and gingivitis, but only in the short term[9]. Hyaluronic Acid (HA) is among the few natural ingredients which has gained wide attention because of its anti-inflammatory, bacteriostatic and antioxidant properties[10]. Invitro and invivo studies demonstrate that HA has a protective effect on oral mucosa due to its viscoelastic properties and also inhibits plaque growth[8]. We could find only one study that compared the effectiveness of HA against the reference standard CHX mouthrinse for its antiplaque and anti-gingivitis potency among healthy subjects in a 4-day plaque regrowth model[10].

Few authors have suggested using a combination of molecules to facilitate synergistic action[8]. Owing to the anti-inflammatory effect of HA and the long term effectiveness combined with less associated adverse events of CPC, a novel mouthrinse combining these molecules was formulated. The present study aims to compare the effectiveness of a CPC-HA based mouthrinse against CHX and placebo (hydro-alcohol based) mouthrinses on plaque and gingivitis in healthy young adults with a good oral health and no prior pathological conditions. Calculus, extrinsic stains, oral malodor and adverse events occurrence were considered as the secondary outcomes. It was hypothesized that the new mouthrinse formulation demonstrates the same clinical effect of CHX mouthrinse, and reduced side effects.

Material and methods

Study design

This was a 21-day randomized, double-blind, three arm parallel study with random allocation of subjects to any of the three groups and an allocation ratio of 1:1:1; 0.05% CPC-HA based mouthrinse (Gengyve, CDR Pharma, Milan, Italy), 0.2% Chlorhexidine mouthrinse (Rexidin, Warren, Indoco Remedies Ltd, India) and a hydro-alcohol based mouthrinse which served as placebo. In addition to the mouthrinse, all the participants were provided with fluoridated toothpaste (Colgate Strong teeth, Colgate-Palmolive, India) and toothbrushes (Colgate Sensitive Ultra Soft, Colgate-Palmolive, India). Thorough dental prophylaxis for removal of plaque, calculus and stains was provided to each participant by qualified dental professionals at baseline which was followed by baseline examination. All the participants were examined for oral malodor, stains, calculus, gingivitis and dental plaque by a single calibrated examiner (VVB), a dental public health postgraduate student, who was blind to the allocation status of the participants. The same examiner assessed the participants again at the end of the experiment. Reporting of this study conforms to the Consolidated Standards of Reporting Trials guidelines[11]. The protocol of this clinical trial was registered with the Clinical trial registry of India (CTRI/2018/02/012054) and can be accessed here (http://ctri.nic.in/). There were no deviations from the initial protocol after the commencement of the study.

Study population

Fourth year dental students of a dental institution in India were invited to participate. All those expressing interests were provided with an information sheet and were explained about the aims and methods of the study. Those willing to participate signed written informed consents. Ethics approval was obtained from the institutional ethics committee. All the students with a full set of dentition, practicing regular oral hygiene and agreeing to provide

informed consent were considered for inclusion while the exclusion criteria were as follows

- People who have undergone periodontal treatment within the last three months
- Subjects who have used mouthrinses, local or general medication within the last month
- Subjects who are allergic or sensitive to mouthrinses ingredients
- Those with systemic and chronic diseases
- Those with periodontal pockets deeper than 4 mm around teeth
- Individuals undergoing orthodontic treatment including removable maintenance appliances
- Individuals with extensive intrinsic teeth staining
- Regular smokers and alcohol consumers.

All those willing to participate underwent a screening examination for eligibility and were interviewed about their smoking and alcohol consumption status. This study was conducted at the postgraduate clinic of Department of Public Health Dentistry, SRM Dental College, Chennai, India.

G-power was used to calculate the required sample size for the two primary outcomes of the study. Differences in means of the primary outcomes (plaque and gingivitis scores) from a previous study that compared the effectiveness of HA with CHX and placebo in preventing dental plaque and gingivitis were used to calculate cumulative effect sizes using the means and Standard Deviation values reported for the three mouthrinse groups[10]. With effect sizes of 0.50 and 0.33 for plaque and gingivitis respectively, a sample of 30 subjects (10 in each group) for plaque and 60 (20 in each group) for gingivitis was considered adequate with 80% power and 5% type I error. The required sample size was therefore 60 subjects. A sample size of 25 in each group was considered adequate to account for patient attrition. Both effect size values correspond to a medium clinical effect.

Clinical evaluation

The primary outcomes of this study were plaque accumulation and the extent of gingivitis. Calculus, extrinsic teeth staining, oral malodor and adverse events served as the secondary outcomes. All the clinical indices were recorded in the order described below. Oral malodor was recorded using organoleptic assessment. The examiner sniffed patient's breath at a distance of 20 cm as they expired the air by mouth after deeply inspiring by nostrils. Oral malodor was assessed on a six-point intensity rating (0=No odor, 1=Slight odor, 2= moderate odor 3=Heavy odor, 4=Strong odor, 5=Intense odor)[12] which was re-categorized as no odor (score 0), slight (scores 1-2) and heavy (scores 3-5). Tooth staining was assessed using Lobene stain index[13], which involves the examination of eight incisors (the nearest canine is examined when an incisor is missing). Assessment for intensity (0=no stain, 1=light stain, 2=moderate stain and 3=heavy stain) and the extent (0=no stain, 1= stain on 1/3rd area, 2=stain covering 1/3rd to 2/3rd area, 3=stain covering >2/3rd area) was done on facial and lingual surfaces of all the incisors on two regions (tooth surface is divided into two regions; gingival area and the remaining tooth surface area called body of the tooth surface). Calculus accumulation of teeth surfaces was quantified using Volpe-Mannhold index[14] with the help of a graduated periodontal probe. For this purpose, lingual surfaces of the six lower anterior teeth are measured in millimetres in three planes (mesiolingual, midlingual, and distolingual). The distances measured (18 measurements per subject) were totalled and represented the Volpe-Mannhold Index for each subject. The gingival examination was done using Gingivitis by Loe-Silness index[15]. It includes an examination of four surfaces (mesial, distal, buccal and lingual) on six index teeth (16, 12, 24, 36, 32, 44) for gingival inflammation and bleeding on probing. The gingival inflammation scores range from 0 (normal gingiva) to 4 (severe inflammation marked by redness and edema/ulceration/tendency to bleed spontaneously). The total gingival index score for each subject was calculated by adding all the individual

scores on four surfaces of each tooth and dividing this sum by the total number of surfaces assessed. Assessment of plaque was done using a Turesky modification of Quigley Hein index[16]. To assess the plaque accumulation, each tooth (except the third molars) was scored in six areas (mesiofacial, midfacial, distofacial, mesiolingual, mid-lingual, and distolingual) after disclosing the plaque using an Erythrosine based disclosing agent (Plakcheck, Vishal dentocare, India). Score for plaque accumulation on each tooth surface can range from 0 (no plaque) to 5 (plaque covering two-thirds or more of the tooth surface). The Plaque index score for each subject was calculated by adding all the individual scores for each tooth and dividing this score by the total number of surfaces assessed.

In addition to these clinical parameters, the oral mucosa of each subject was examined for any abnormalities. Subjects were also interviewed at their follow-up examination if they have encountered any adverse events during the experiment. All the participants were instructed not to eat or drink anything at least for an hour before the clinical evaluation and also to avoid alcoholic drinks, carbonated beverages, foods containing onion, garlic or spices 8 hours before the clinical assessment.

Randomization

All the subjects willing to participate were assessed for eligibility by another examiner, those eligible provided written informed consent. A sequence of random numbers was generated by one of the investigators (SK) using SPSS and each participant received a number in a sealed envelope from a coordinator who was not involved in the data collection. Each subject was assigned a kit which was numbered sequentially using a randomly generated list of numbers by another assistant. Each kit consisted of a mouthrinse, toothbrush and toothpaste. All the mouthrinses were placed in similar coloured containers to facilitate double blinding. The participants were advised only to use those oral hygiene products that were provided to them

throughout the experimental period of 21 days, they were also instructed not to use any interdental oral hygiene aids during the experimental period. All the subjects were advised to brush twice every day with the given toothpaste and toothbrush and rinse with 15 ml of mouthrinse twice every day for 30 seconds, 30 min after morning and night brushing throughout the experimental period. Dispensing cups measuring 15 ml were also provided to the participants. Verbal instructions on the technique of brushing and the procedure of mouthrinse use were delivered to all participants by the study coordinator.

Statistical analysis

Descriptive statistics were computed for each analysed variable within each study group; the normality of the distributions was assessed by Shapiro Wilk test. As all primary and secondary outcomes data were not normally distributed, median and interquartile range was used as the descriptive measure. In addition, means and standard deviations were presented for ease of interpretation. To compare gender, oral malodor and adverse events occurrence by the group, we used Chi-square test. One way ANOVA was used to compare age of the participants between the experimental groups. Kruskal Wallis test was used to compare clinical indices values at baseline and change in the clinical status among groups; statistical power was estimated as an adjustment of one way ANOVA. Post hoc comparisons were done by Mann Whitney test; Bonferroni correction was applied. Wilcoxon test for paired data was used to compare clinical indices values between the baseline and follow up in each group separately. A p-value of <0.05 was considered statistically significant. All analyses were done using Stata 14.2.

Results

All the fourth year dental students (N=90) were invited to participate (Figure 1). Of which, 81 showed interest, but six were excluded due to various reasons. A total of 75 subjects were randomised between October to December 2017 and all of them completed both baseline and follow-up examinations. There was no attrition and each group comprised 25 participants. The age range of the subjects was 20-23 years (Mean: 20.7 years; SD: 0.78) with a majority (75%) being females. There were no regular smokers or alcohol consumers, 8 (11%) and 12 (16%) participants reported of smoking and consuming alcohol at least once in their lifetime respectively. At baseline, there were no significant differences in age and gender distribution between the test groups (Table 1).

Primary outcomes

At baseline, there were no differences in plaque and gingivitis scores between the three groups (Tables 2 and 3); most of the patients in all the groups were free of gingival inflammation and plaque accumulation. There was an increase in plaque accumulation in all the three groups at 21-day follow-up; CPC-HA $p=0.002$, CHX $p=0.005$, placebo $p<0.001$ (p values of post-hoc tests are not presented in tables). Table 2 demonstrates that there was a significant difference for change in plaque index scores between the groups (estimated power 80%) with subjects in the placebo group experiencing higher levels of plaque accumulation (mean±SD: 0.047±0.05) than the test (0.015±0.02) and positive control (0.01±0.02) groups. On post hoc comparisons after Bonferroni correction, no difference was observed between the CPC-HA and CHX groups ($p=0.942$).

Table 3 demonstrates that similar to plaque accumulation, gingival inflammation increased in all the experimental groups (CPC-HA p=0.015, CHX p=0.015, Placebo p<0.001) but there were no differences for change in gingival index scores between the three experimental groups (estimated power 70%).

Secondary outcomes

At baseline, no inter-group differences in the clinical parameters were observed; all the patients were free of calculus (Table 4). However, there were few subjects with teeth staining (Table 5). There was an increase in teeth staining in CHX (mean±SD: 2.6±3.0, p<0.001) and placebo groups (2.32±3.30, p=0.002) while such finding was not observed in CPC-HA users (0.16±1.46, p=0.573) after 21-days of mouthrinse use. Although there was a significant difference between the three groups (p=0.004; estimated power 75%) for change in teeth staining, no differences were observed between the pairs of groups on posthoc comparisons. No differences were found between the three experimental groups for change in calculus scores (Table 5; estimated power 36%). Table 6 shows that there was no difference in the frequency of oral malodor between the groups.

A total of 21 adverse events were registered, 5 (20%) among CPC-HA users, 10 (40%) in CHX users and 6 (24%) among users of the placebo group. There was no significant difference between the groups, and there were no patients who reported more than one adverse event (Table 6). The most frequently reported adverse event was an ulcer in CPC-HA group, tongue staining in CHX and Dysguesia in placebo group. No mucosal abnormalities were observed on clinical examination of oral mucosa in any of the patients.

Discussion

This is the first instance where a new formulation that combined two molecules (CPC and HA), each of which were proven to be effective in prevention of plaque and gingivitis, has been tested. HA is a non-surface active water soluble anionic polyelectrolyte with anti-inflammatory, bacteriostatic and anti-oxidant properties which could prevent the cytotoxic effects of the cationic surfactants (CPC) on human cells[17, 18]. The combination of HA and cationic surfactants have been widely studied during the recent times for its antimicrobial properties[19]. There have been several studies that evaluated the effectiveness of HA alone in various forms in improving gingival and periodontal status[20]. Most of the studies have reported promising results and HA has also been used in postoperative care[21]. However, the role of mouthrinses containing HA with any combination has not been adequately studied in healthy patients as a regular chemical plaque control measure. In order to facilitate synergistic effectiveness, a combination of two promising molecules was used. HA forms a coating in the oral cavity[21] thus protecting the oral mucosa while CPC causes bacterial inactivation with staining as the only side effect.

Although some authors suggested implementation of a pre-experimental phase comprising supervised oral hygiene prior to the initiation of experimental gingivitis model[22], a pre-experimental phase was not considered essential in this 21-day study. This is because, dental students who constitute the study sample had very good oral hygiene which could be ascribed to their knowledge and self-efficacy for practising oral hygiene that they have gained through their curriculum. Also, a thorough dental prophylaxis was provided which is demonstrated through the extremely low plaque and gingival index scores observed at baseline. Van der Weijden et al., compared the impact of the length of pre-experimental period and observed that this duration did not have any effect. Instead, individual variations between the subjects were observed and thus it is recommended that all gingivitis

experimental studies should use a sufficient number of subjects to account for individual variation[23]. The sample size used in this study was calculated to obtain an adequate statistical power according to a previous investigation where the same active had been used [10]. Further, there were no subjects lost to follow-up; attrition in experimental research is considered to cause serious problems[24].

At 21-days follow-up, there were no differences between the CHX and CPC-HA groups for plaque accumulation which demonstrates that CPC-HA combination is as effective as CHX in preventing plaque. Direct comparison of our study findings with past literature is not possible as there are no studies that used the novel CPC-HA mouthrinse. However, data from the only study that used the HA mouthrinse[10] indicates that the mean plaque scores, measured by Turesky modification of Quigley Hein index, in HA group was 0.41 while that in the CHX and placebo groups was 0.35 and 0.80 respectively. Our findings also indicate that the mean plaque scores at 21-days follow-up in CPC-HA and CHX groups were approximately similar with 0.015 and 0.01 respectively while the mean score in the placebo group was 0.047.

Moreover, there were no differences between the groups for gingival index scores and this finding is in agreement with the past HA mouthrinse study that reported no significant differences in gingival bleeding, measured by sulcular bleeding index, between the HA, CHX and placebo groups[10]. This finding in our study could be attributed to the shorter duration of the study and use of regular oral hygiene aids by the participants during the experimental period. Although the 21-day experimental gingivitis model requires the participants to withdraw all active efforts required to maintain good oral hygiene [1], it causes discomfort to the participants. Also due to ethical concerns, all the subjects were allowed to perform regular oral hygiene practices using fluoridated toothpaste and ultrasoft toothbrushes in the current investigation. To maintain consistent practices between the subjects belonging

to different experimental groups, they were provided with toothbrushes and toothpaste. Plaque accumulation and gingivitis scores increased in all the groups at the end of the experimental period. Studies performed a few decades ago using experimental gingivitis model observed that without the use of other oral hygiene measures, plaque and gingivitis developed in individuals using either an antimicrobial mouthrinse or a placebo[25]. Histopathological studies suggest that inflammatory infiltrates accumulate in connective tissue around the pocket epithelium in those areas of the tooth that are not cleaned during the experimental period, and the gingival conditions are related to the compositions of plaque[26].

Tooth staining was observed in both CHX and the placebo groups but not in CPC-HA group. Both placebo and CHX contained an edible colouring agent. Staining of the tongue was observed in 12% of CHX users but there were no significant differences between the groups for the occurrence of adverse events or oral malodor. A systematic review has found that staining was the most commonly reported adverse event in most of the CHX users. Although calculus formation was associated with CHX use in few studies[27], we did not find any differences between the mouthrinses for calculus formation which might be due to the short duration of this trial. There were some sporadic reports of ulcers, dysguesia, altered taste, dryness and oral itching. This demonstrates that the self-reported adverse events were not related to the use of any of the mouthrinses.

Owing to these side effects, CHX is not indicated for long-term use; alternative mouthrinses have been widely recommended for long-term use. Some of these molecules include essential oils and CPC[8]. A HA alone mouthrinse has also been observed to have anti-oedematous effect in early wound healing in patients with dental implants[28]. Several studies reported the anti-inflammatory effect of HA-based gel in patients with gingivitis, periodontitis, dental implants[29-32]. Because of these advantages coupled with its effectiveness in plaque reduction that is comparable to CHX, CPC-HA based mouthrinse could serve as a

promising alternative for regular home-based chemical plaque control measure. Moreover, by combining a high molecular weight natural compound with a well-known antiplaque chemical agent, we can obtain a formula with a synergistic effect. The promising effect of this combination, as shown in this short term study, exploits the positive properties of both the actives, reducing the intrinsic side effect of the mouthrinse. However, the long-term effectiveness of this combination is yet to be evaluated. As suggested by the Council of Dental Therapeutics criteria[33], studies should last for a minimum duration of 6 months. Therefore, we are planning to conduct a six months study to assess the effectiveness of CPC-HA mouthrinse in comparison to the reference standard mouthrinse (CHX). Also, we intend to compare the effect of CPC-HA with HA alone to confirm the synergistic effect of CPC and HA over the use of HA alone.

Additionally, our aim was to investigate the effect of the new mouthwash formulation in a group of healthy subjects, who had a baseline good oral health. In this population, alteration in clinical measures (gingivitis, plaque, etc) would more probably be caused by the experimental treatment than by a prior pathological condition. Also, they should represent a possible target population for the new mouthwash formulation that could be proposed as long term maintenance treatment also in healthy subjects without specific clinical needs[8, 10]. Future studies will focus on selected groups of patients with oral alterations: they will test the improvement in clinical indices and the possible therapeutic effect of the mouthwash.

There are some limitations in this study which needs mentioning; compliance was not assessed, dental students were the study population, who are more likely to comply with the oral hygiene instructions, but might not represent the typical patient population, thus limiting the external validity of the study findings. Another limitation is that the study design deviated from the traditional experimental gingivitis model where the subjects practised their regular oral hygiene procedures in addition to the use of test mouthrinses. These limitations could

have attenuated the antiplaque and antigingivitis effect of the test mouthrinses.

In conclusion, CPC-HA and CHX mouthrinses had similar effectiveness in preventing plaque accumulation while no differences were observed between the mouthrinses for preventing gingivitis. Dental staining increased in CHX and placebo mouthrinse users but not in those using CPC-HA. No differences were observed between the experimental groups for prevention of calculus and oral malodor. Fewer subjects belonging to CPC-HA mouthrinse group reported adverse events compared to CHX, however this was not statistically significant.

Clinical relevance

Scientific rationale for the study - The most effective mouthwash agent is CHX, but it is associated with adverse effects. HA, a natural compound with anti-inflammatory, bacteriostatic and antioxidant properties, was combined with CPC, an antiplaque chemical agent.

Principal findings – After 21 days, CPC-HA and CHX mouthrinses similarly prevented plaque accumulation. CHX and the placebo mouthrinses caused dental staining but not CPC-HA.

Practical implications - The CPC-HA combination is as effective as CHX in preventing plaque. The synergistic activity of the molecules resulted in promising short-term effects, reducing the side effects of the mouthrinse. The long-term effectiveness should be evaluated.

Source of support

This study was funded by CDR Pharma, Milan, Italy that provided the Gengyve mouthwash, used in the current study. However, the funding organization did not have any role in data collection and presentation of the results.

Conflict of interest

CDR Pharma (Milan, Italy) provided the Gengyve mouthwash to be used in the current study.

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Table 1: Demographic data

	CPC-HA	Chlorhexidine	Placebo	Total	Significance
Male (n, %)	6 (24%)	6 (24%)	7 (28%)	19 (25.3%)	1.00*
Age (mean, sd)	20.6 (0.8)	20.6 (0.9)	20.8 (0.7)	20.7 (0.78)	0.665**

** Chi Square test*

*** One way ANOVA*

Table 2: Plaque index at baseline and 21-days follow-up in each group (n= 25 for each group)

	CPC-HA		**Chlorhexidine**		**Placebo**		**P***
	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)	Mean (SD)	
At baseline	0 (0-0.01)	0.005 (0.01)	0 (0-0.01)	0.005 (0.01)	0 (0-0.01)	0.007 (0.01)	0.866
At 21 days	0 (0-0.02)	0.02 (0.02)	0 (0-0.01)	0.015 (0.02)	0.04 (0-0.09)	0.054 (0.05)	0.015
Change	0 (0-0.02)	0.015 (0.02)	0 (0-0.01)	0.01 (0.02)	0.04 (0-0.09)	0.047 (0.05)	0.015

** Kruskal Wallis test*

Table 3: Gingival index scores at baseline and 21-days follow-up in each group (n= 25 for each group).

	CPC-HA		Chlorhexidine		Placebo		P*
	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)	Mean (SD)	
At baseline	0 (0-0)	0.012 (0.03)	0 (0-0)	0.005 (0.02)	0 (0-0)	0 (0.0)	0.754
At 21 days	0 (0-0.00)	0.037 (0.06)	0 (0-0.04)	0.038 (0.07)	0.04 (0-0.17)	0.087 (0.10)	0.080
Change	0 (0-0.00)	0.025 (0.06)	0 (0-0.04)	0.033 (0.06)	0.04 (0-0.17)	0.087 (0.10)	0.080

** Kruskal Wallis test*

Table 4: Extrinsic stains on teeth surfaces at baseline and follow-up (n=25 in each group)

	CPC-HA		Chlorhexidine		Placebo		P*
	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)	Mean (SD)	
At baseline	1 (0-1)	0.88 (0.93)	0 (0-1)	0.72 (0.89)	1 (0-2)	0.88 (0.83)	0.720
At 21 days	0 (-1-1)	1.04 (1.10)	2 (0-4)	3.32 (2.98)	2 (0-3)	3.20 (3.08)	0.004
Change	0 (-1-1)	0.16 (1.46)	2 (0-4)	2.6 (3.0)	2 (0-3)	2.32 (3.30)	0.004

** Kruskal Wallis test*

Table 5: Calculus index scores at baseline and 21-days follow-up in each group (n= 25 for each group)

	CPC-HA		Chlorhexidine		Placebo		P*
	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)	Mean (SD)	
At baseline	0 (0-0)	0.12 (0.30)	0 (0-0)	0.10 (0.25)	0 (0-0)	0.12 (0.26)	0.970
At 21 days	0 (0-0)	0.18 (0.45)	0 (0-0)	0.16 (0.55)	0 (0-0.5)	0.38 (0.65)	0.494
Change	0 (0-0)	0.06 (0.58)	0 (0-0)	0.06 (0.63)	0 (0-0.5)	0.26 (0.72)	0.494

** Kruskal Wallis test*

Table 6: Oral malodor at baseline and 21-days follow-up and total adverse events reported by the subjects (each group contains 25 subjects).

	CPC-HA n (%)	CHX n (%)	Placebo n (%)	P*
Adverse events				
At least one Adverse Event	5 (20%)	10 (40%)	6 (24%)	0.249
Ulcer	2 (8%)	1 (4%)	1 (4%)	1.000
Dysgeusia	1 (4%)	2 (8%)	2 (8%)	1.000
Tongue staining	1 (4%)	3 (12%)	1 (4%)	0.609
Altered taste	1 (4%)	1 (4%)	0	1.000
Dryness	0	1 (4%)	1 (4%)	1.000
Oral itching	0	2 (8%)	1 (4%)	0.769
Oral malodor (baseline)				
No odor	25 (100%)	24 (96%)	23(92%)	0.769
Slight odor	0	1 (4%)	2 (8%)	
Heavy odor	0	0	0	
Oral malodor (21 days)				
No odor	23 (92%)	22 (88%)	23(92%)	
Slight odor	1 (4%)	3 (12%)	2 (8%)	0.870
Heavy odor	1 (4%)	0	0	

**Chi Square test*

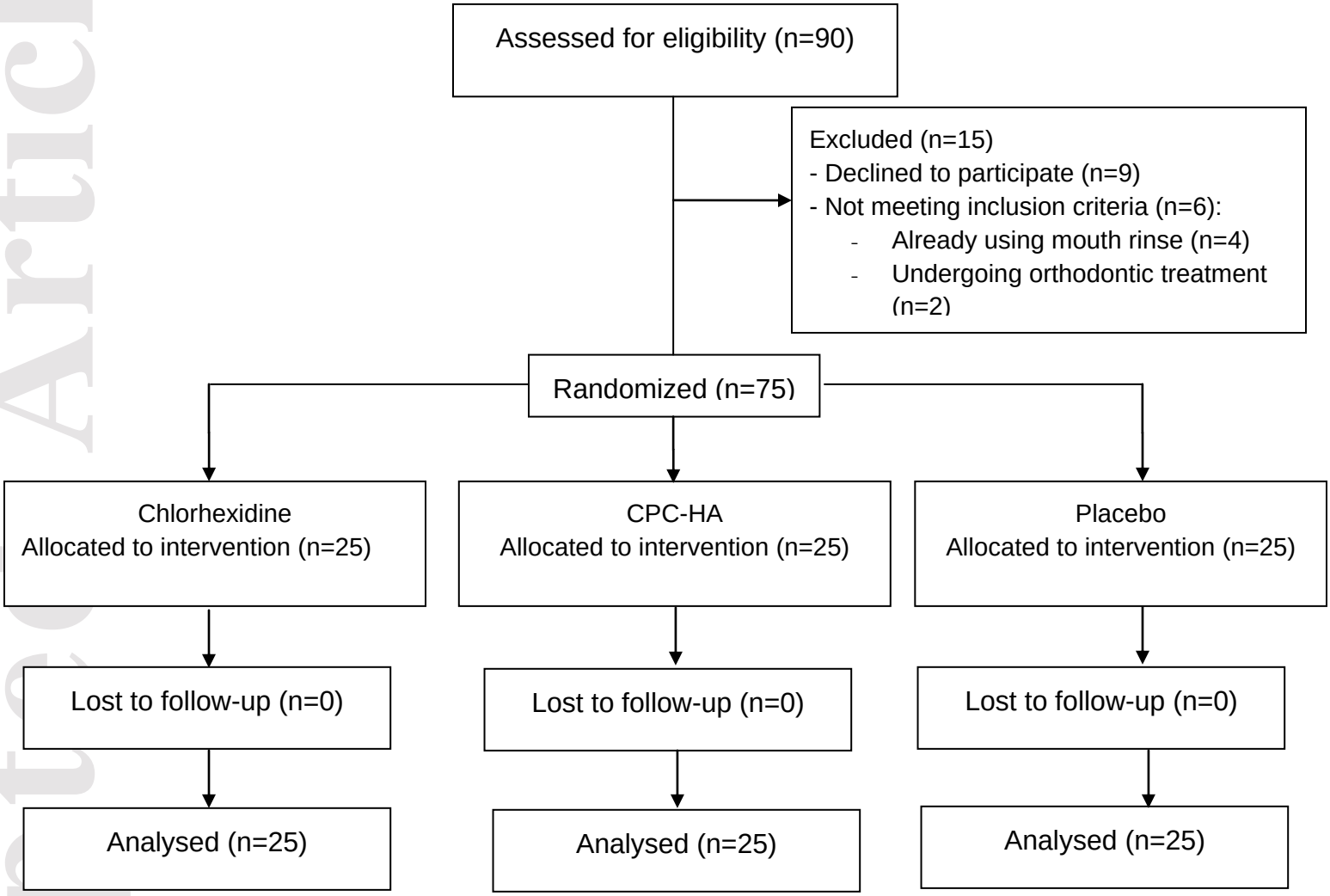

Figure 1: CONSORT flowchart depicting the participant recruitment