Clinical and microbiological effects of commercially available dentifrice containing amine fluoride: A randomized controlled clinical trial

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Abstract

Background: The inability of the normal adult population to perform adequate tooth brushing has led to the search for chemotherapeutic agents in order to improve plaque control. This 6 month, single center, randomized controlled clinical trial was conducted to assess the clinical and microbiological effects of a dentifrice containing only amine fluoride (AF) as the active ingredient on gingivitis. **Materials and Methods:** Ninety subjects diagnosed with chronic generalized gingivitis were selected and randomly divided in three groups: Group 1 – placebo toothpaste, Group 2 – AF containing toothpaste, and Group 3 – triclosan containing toothpaste with polymer and fluoride. Clinical evaluation was undertaken using the gingival index of Loe and Silness and the plaque index and microbiological counts were assessed at baseline, 6 weeks, 12 weeks, and 24 weeks. A subjective evaluation was also undertaken by a questionnaire. **Results:** AF containing toothpaste showed significant improvement in gingival and plaque index scores as well as microbiologic counts compared with placebo dentifrice. These improvements were comparable to triclosan containing toothpaste. **Conclusions:** AF containing toothpaste may be a useful formulation for chemical plaque control agent and improvement in plaque and gingival status and add to the list of various therapeutic agents used for maintenance of gingival health.

Keywords: Antiinflammatory agent, antiplaque agent, clinical trial, gingivitis

Introduction

Periodontal diseases encompass multifactorial diseases involving bacterial biofilms and the generation of an inflammatory response, including the production of cytokines, eicosanoids, and matrix metalloproteinases. Bacterial biofilms have been shown to be the primary etiological factor in the initiation of gingival inflammation and subsequent destruction of periodontal tissues.[1] It is well established that supragingival plaque is the cause of gingivitis and plays a primary role in the initiation of periodontitis.[2] The removal of microbial plaque leads to resolution of gingival inflammation, and cessation of plaque control leads to a recurrence of inflammation.

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The control of plaque in the maintenance of gingival health has been well established in the literature.[3,4] It has been shown that rigorous self-performed plaque control over long periods of time reduced the levels and altered the composition of subgingival bacteria and reduced the frequency of deep periodontal pockets.[5,6] The inability of the normal adult population to perform adequate tooth brushing has led to the search for chemotherapeutic agents in order to improve plaque control.[7] These chemicals, mainly triclosan and chlorhexidine, have been used as mouth rinses or added to dentifrices to avoid plaque formation and development of gingivitis.[7-9]

Amine fluorides (AFs) as caries inhibitory and antiplaque agents have been developed about 40 years ago,[10] and their beneficial effect has been proved in over 600 studies from the beginning of the early 70s.[11]

The AF N-octadecyltrimethylendiamine-N, N, N-tris (2-ethanol)-dihydrof luoride ($C_{27}H_{58}N_2O_3$.2 HF) is a cationic antimicrobial known to reduce plaque formation and prevent caries formation.[12]

Toothpaste containing both AF and stannous fluoride (SnF_2) was introduced in 1985 and the cariostatic potential has been documented in several studies.[13] SnF_2 also has a well-known plaque inhibiting effect and may inhibit bacterial metabolism.[14] It has been shown that a combination of AF and SnF_2 reduced both plaque and retarded gingivitis.[15]

However, there have been no recent studies in which the clinical and microbiological effects of a dentifrice containing

only AF as the active ingredient on plaque and gingivitis. Therefore, considering the aforementioned findings, this 6 month, single center, randomized controlled clinical trial was conducted to assess the clinical and microbiological effects of a dentifrice containing only AF as the active ingredient.

Materials and Methods

Ethical approval was obtained from the institutional Ethical committee and Review board. A total of 90 dentate subjects (45 males, 45 females, mean age 30.23 years) who reported to the Department of Periodontics, Government Dental College and Research Institute, Bangalore were recruited for this double-blinded, parallel, randomized controlled clinical trial conducted in May 2011– to November 2011. All randomly screened participants were informed about the nature of the study and signed an informed consent form. Group sample sizes were decided by power analysis with 95% power and a significance level of 0.05.

Subjects diagnosed with chronic generalized gingivitis, aged 25–40 years, having at least 20 natural teeth, with no history of periodontal therapy or previous use of antibiotics or antiinflammatory medication within the preceding 6 months were included in the study. All patients fulfilled the clinical criteria of the gingival index (Loe and Silness[16]) >1, pocket probing depth ≤3 mm, clinical attachment loss=0, with no evidence of radiographic bone loss. Subjects with known allergies to the constituents of the formulation, hematological disorders or other systemic illness, pregnant and lactating females, undergoing orthodontic treatment and with smoking habits were excluded.

The participants were assigned randomly by a computer generated numbering sequence to one of the three groups (30 subjects in each group): Group 1: Placebo toothpaste (Group Pharmaceuticals, Bangalore, India), Group 2: Test toothpaste containing AF (Group Pharmaceuticals, Bangalore, India), and Group 3: Fluoridated toothpaste containing triclosan and polymer (Group Pharmaceuticals, Bangalore, India).

Patients accepted to participate in the study returned for a baseline examination. Patients were told not to perform any oral hygiene (including chewing gum) for 8 h prior to the baseline and follow up examinations. Patients were assessed for plaque using the plaque index (PI) (Tureskey *et al.* modification of Quigley Hein Index) and gingival inflammation using the gingival index (GI) (Loe and Silness), as well as for oral soft-tissue status. Following the assessments, all subjects received a supragingival prophylaxis and polishing to remove plaque, calculus, and extrinsic stain. After prophylaxis, patients were instructed on the proper brushing technique and were given either of the dentifrices or the placebo toothpaste along with a diary to record product usage and daily oral hygiene activities. The dentifrices were dispensed to subjects by a dental assistant not involved in the study. All tubes were covered in plain white covering labeled only with lot numbers to ensure proper blinding of the product from the patients and the examiner. Subjects were also given a soft bristled toothbrush to use during the clinical study. Subjects were asked to refrain from all other unassigned forms of oral hygiene, including nonstudy toothbrushes or toothpastes, dental floss, chewing gum, or oral rinses during the study. Subjects were assessed for gingivitis using the GI and for plaque, using the PI in the same dental unit under identical conditions at baseline, 6, 12, and 24 weeks.

At the baseline and at each visit, the dental plaque sample was collected from each subject. Each volunteer was asked to gargle his or her mouth with saline to remove any food debris. Taking all aseptic measures, the plaque was collected from the buccal groove of the lower first molar tooth using a sterile paper point so that the standardized length of the paper point (colored area) touched the tooth for 5 s. This specimen was immersed in 1 ml of phosphate buffered saline (PBS). These plaque specimens were vortexed for 10 s and immediately subcultures were performed on Mitis Salivarius (MS) agar for streptococcus species and GMC medium for *Actinomyces* species taking 5 ml of plaque in PBS.

The colonies of *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus intermedius, Streptococcus orali, Actinomyces viscosus,* and *Actinomyces naeslundii* were identified based on colony morphology. The colonies with similar morphology were counted using a colony counter and their numbers were recorded and the total number was taken into account. Apart from clinical and microbiologic evaluation, subjective evaluation was also undertaken at each visit, using a questionnaire relating to the taste and flavor of the dentifrices or any adverse effect experienced after use. To check for compliance, the participants were asked to return their assigned tubes, so that the investigator could verify the amount of dentifrice that was used.

Statistical analyses

Analysis of data was carried out using SPSS version 10.5 (SPSS, Chicago, IL). The values of different parameters collected are expressed as means ± standard deviation (SD). Normality of continuous data was tested using Kolmogorov–Smirnov test. Mean change from baseline to 6, 12, and 24 weeks were calculated. Comparison between the three treatment groups and within each treatment group was performed using one way analysis of variance (ANOVA). Posthoc comparisons were made using Scheffe test if the ANOVA statistics was significant at 0.05 levels.

Results

Seven subjects did not complete the study and were excluded from the analysis [Figure 1]. There was no significant

difference between groups 1, 2, and 3 with respect to PI and GI scores at baseline. There was a gradual decrease in the PI and GI scores by 6, 12, and 24 weeks time interval, respectively, in all three groups [Table 1].

Mean change in all the parameters is also given in Table 2. Intra group comparison for all the parameters is given in Table 3. A significant difference was observed in PI, GI, and microbial counts in all the groups at all the time intervals.

Inter group comparison of mean change at various intervals is shown in Table 4. No significant difference was found between groups 2 and 3 for any parameter. However, significant difference was found with respect to reduction in PI, GI, and microbial counts in group 1 as compared with groups 2 and 3 at the end of 24 weeks [Table 4].

On subjective evaluation, all the subjects gave positive responses regarding the taste and flavor of all the dentifrices.

Discussion

While the mechanical control of dental plaque has been clearly shown to retard the advance of gingivitis and periodontal disease,[3,17] Axelsson and Lindhe reported that noncompliant patients exhibited signs of recurrent disease processes.[3] Owing to the inconsistency of simple mechanical control of plaque accumulation, a number of chemotherapeutic agents have been incorporated into home use products to control plaque and gingivitis. These agents have generally been incorporated into either mouth rinses or toothpastes. The main action of these agents has been focused on their antimicrobial action. There have been a number of active ingredients incorporated into various dentifrices. Triclosan/copolymer dentifrices have been studied extensively for their antiplaque and antigingivitis effectiveness. Tricolsan is a phenolic agent comprised of bisphenol and a nonionic germicide.[18] Lindhe *et al.* reported on the results of a 6-month clinical trial comparing a triclosan/copolymer dentifrice with a fluoride-containing dentifrice and found that the triclosan group had more plaque reduction and resolution of gingivitis than the regular fluoride dentifrice group.[19] Studies including long-term clinical trials,[20] short-term experimental gingivitis models,[21] and short-term randomized clinical studies[22] have demonstrated significant reductions in plaque and gingivitis from about 20% to as high as 60%. Considering aforementioned data, triclosan containing dentifrice with copolymer was taken as a positive control in this study.

AFs as caries inhibitory and antiplaque agents have been developed about 40 years ago,[10] and their beneficial effect has been proved in over 600 studies from the beginning of the early 70s.[11]

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The findings of this present study indicate that dentifrice containing AF alone also can be equally efficacious as containing triclosan and polymer in the reduction of plaque and gingivitis.

The reduction in plaque and gingivitis scores in group 1 (placebo) can be attributed to the Hawthorne effect (i.e., patients frequently appear to improve merely from the effects of being placed in a clinical trial).[23]

Table 1: PI, GI scores and microbial counts of all groups at different follow-ups

Group	Baseline	6 weeks	12 weeks	24 weeks
PI scores at baseline and different follow-ups				
1	4.406±0.713	3.523±0.776	3.266±0.831	3.200±0.754
2	4.428±0.777	3.628±0.752	2.912±0.716	2.497±0.685
3	4.417±0.570	3.615±0.713	3.024±0.771	2.509±0.683
GI scores at baseline and different follow-ups				
1	2.002±0.403	1.504±0.400	1.313±0.334	1.288±0.381
2	2.054±0.380	1.241±0.321	0.951±0.284	0.796±0.205
3	1.920±0.386	1.301±0.529	1.017±0.449	0.781±0.363
Microbial counts at baseline and different follow-ups (×1000000)				
1	31.187±1.890	26.153±2.938	23.683±2.910	23.120±3.026
2	30.043±3.806	19.667±3.255	12.568±2.270	9.280±2.045
3	30.603±3.054	21.503±3.136	13.857±2.582	9.982±1.780

Because of its cationic nature, AF are also expected to interact with negatively charged bacterial cell surfaces as well as with salivary pellicle surfaces through electrostatic interactions.[24] AF adsorption to salivary pellicles has been extensively studied in the past[25] with respect to its effects on the charge and hydrophobicity of pellicle surfaces, but never with respect to its effects on tooth surfaces *in vivo,* as can be established through intraoral contact angles measurements.[26] Recent studies have shown that the hydrophobicity of tooth surfaces goes through a daily cycle, becoming hydrophilic after brushing and recovering to relatively hydrophobic values during the day, depending on the type of toothpaste employed.[27] Interestingly, *in vitro* water contact angles on toothpaste-treated and untreated pellicle surfaces were generally more hydrophilic than those observed *in vivo,* likely because *in vivo* greasy substances adsorbed from food and initial biofilm formation form part of the pellicle.[28]

Another unexplored aspect of AF is its antimicrobial mode of action. Plaque formation, like biofilm formation in general, commences with an initial adhesion phase after which the adhering organisms start to grow and form a biofilm.[29] Considering the effects of AF on the pellicle surface

Table 2: Mean change in PI, GI and microbial counts at different follow-ups

Group	BL-6 weeks	BL-12 weeks	BL-24 weeks
Mean change in PI scores at different follow ups			
1	0.883±0.350	1.140±0.530	1.205±0.437
2	0.800±0.547	1.516±0.654	1.931±0.639
3	0.802±0.650	1.392±0.797	1.907±0.736
Mean change in GI scores at different follow ups			
1	0.498±0.307	0.689±0.359	0.714±0.458
2	0.813±0.465	1.103±0.492	1.258±0.388
3	0.618±0.435	0.903±0.484	1.139±0.452
Mean change in microbial counts at different follow ups (×1000000)			
1	5.033±2.201	7.503±2.409	8.067±2.632
2	10.376±2.497	17.475±3.256	20.763±3.760
3	9.100±2.929	16.747±3.225	20.621±3.035

Table 3: Intra group comparison at various follow–ups

Group	Comparison between visits	PI		GI		Microbial count	
		Mean diff.	*P* value	Mean diff.	*P* value	Mean diff.	*P* value
1	BL vs 6 weeks	0.883	<0.001*	0.498	<0.001*	5.033	<0.001*
	BL vs 12 weeks	1.140	<0.001*	0.689	<0.001*	7.503	<0.001*
	BL vs 24 weeks	1.205	<0.001*	0.714	<0.001*	8.067	<0.001*
2	BL vs 6 weeks	0.800	0.001*	0.813	<0.001*	13.701	<0.001*
	BL vs 12 weeks	1.516	<0.001*	1.103	<0.001*	23.075	<0.001*
	BL vs 24 weeks	1.931	<0.001*	1.258	<0.001*	27.416	<0.001*
3	BL vs 6 weeks	0.802	<0.001*	0.618	<0.001*	9.100	<0.001*
	BL vs 12 weeks	1.392	<0.001*	0.903	<0.001*	16.747	<0.001*
	BL vs 24 weeks	1.907	<0.001*	1.139	<0.001*	20.621	<0.001*

*Statistically significant

Table 4: Inter group comparison of mean change at various intervals

Interval	Comparison between groups	Mean reduction from baseline					
		PI		GI		Microbial count	
		Mean diff.	*P* value	Mean diff.	*P* value	Mean diff.	*P* value
BL-6 weeks	3 vs 2	0.002	1.000	–0.195	0.187	–1.276	0.161
	3 vs 1	–0.081	0.840	0.120	0.523	4.067	<0.001*
	2 vs 1	–0.083	0.831	0.315	0.014*	5.343	<0.001*
BL-12 weeks	3 vs 2	–0.124	0.774	–0.200	0.233	–0.728	0.642
	3 vs 1	0.252	0.349	0.214	0.187	9.243	<0.001*
	2 vs 1	0.376	0.099	0.414	0.003*	9.972	<0.001*
BL-24 weeks	3 vs 2	–0.023	0.989	–0.119	0.571	–0.142	0.985
	3 vs 1	0.702	<0.001*	0.424	0.001*	12.554	<0.001*
	2 vs 1	0.725	<0.001*	0.543	<0.001*	12.696	<0.001*

*Statistically significant

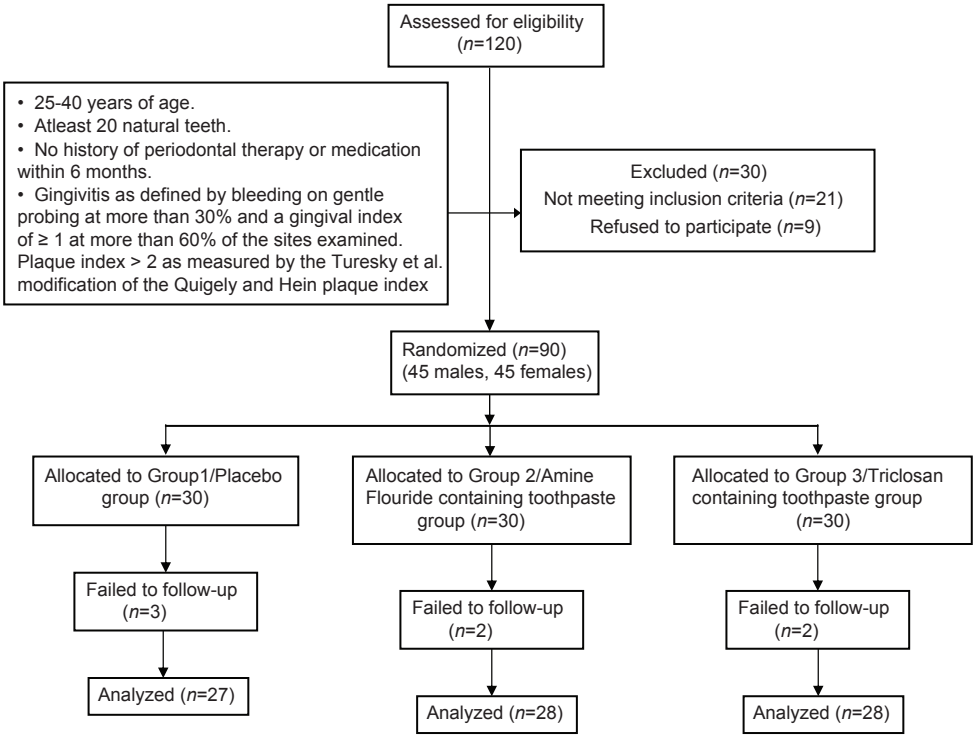

Figure 1: Study flow chart

properties, as well as its known antimicrobial properties, its mode of action could either be through effects on bacterial adhesion, bacterial growth or killing.

Further studies are required to explore the various mode of action through which this agent can play crucial role in the management of periodontal diseases.

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