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Efficacy of 0.75% Boric Acid and 0.2% Chlorhexidine Subgingival Irrigation as an Adjunct to Scaling and Root Planing In the Treatment of Gingivitis. A Comparative Clinico-Microbiological Study

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Keywords

Anti-infective agents; Boric acid; Chlorhexidine; Gingivitis; Root planning

Abstract

Aim: Boric acid has been reported to have antibacterial and anti-inflammatory properties. The purpose of this study was to evaluate the effects of 0.75% boric acid irrigation as an adjunct to scaling and root planing on clinical and microbiological parameters and compare this method with 0.2% chlorhexidine and saline irrigation in patients with chronic gingivitis.

Methods: 60 patients were recruited and divided equally into three groups: I. Saline group, II.0.2% chlorhexidine group and III. 0.75% boric acid group. Subgingival plaque samples were collected after supragingival scaling, prior to subgingival irrigation and processed immediately for analysis of *Porphyromonas gingivalis* and *Prevotella intermedia*. At baseline, 2 weeks and one month after SRP, clinical parameters including plaque index, gingival index, sulcular bleeding index, probing pocket depth were assessed along with the subgingival plaque sample.

Results: Significant reduction in clinical parameters and mean levels of *Porphyromonas gingivalis* and *Prevotella intermedia* were noted at 2 weeks and one-month in all treatment groups. A significantly greater mean reduction of *Porphyromonas gingivalis* was found in the 0.75% boric acid group.

Conclusion: 0.75% boric acid irrigation could be considered as an adjunct to scaling and root planning in the treatment of patients with moderate to severe gingivitis.

Introduction

Periodontal diseases are inflammatory conditions of infectious nature. The unequivocal role of dental bacterial plaque in the development of these diseases was established almost 40 years ago [1]. Although, development of gingivitis after plaque accumulation appears to be a universal finding, the rate of development and the degree of the clinical inflammatory response is variable between individuals, even under similar plaque accumulation conditions.

It is generally accepted that the goal of initial periodontal therapy is to restore the biological compatibility of periodontally diseased root surfaces. While non-surgical periodontal therapy aims to eliminate pathogenic bacteria in the bio films, complete elimination remains elusive. Meticulous subgingival debridement is inherently time-consuming and difficult procedure that usually includes scaling and root planning by manual instrumentation and/or periodontal debridement with sonic or ultrasonic scalers [2]. However, its success relies heavily on the skill of the clinician.

Since most patients are not skilled in adequate plaque removal, many clinicians currently include one or more adjunctive chemotherapeutic agents in their nonsurgical anti-infective regimen. The primary objective of supragingival irrigation is to flush away bacteria coronal to the gingival margin, thereby diminishing the potential of developing gingivitis or decreasing existing gingival inflammation. In contrast, subgingival irrigation attempts to directly reduce the pocket microflora to prevent initiation of periodontal diseases or to facilitate their reduction. Therefore, procedures of eliminating periodontal pathogens are of great interest, and considerable attention has been devoted to the possibility of using antibiotics or antiseptics in this respect [3]. Antimicrobials, including povidone-iodine and chlorhexidine, have been used with limited success in the treatment of periodontal diseases as a result of potential toxicity and the unique anatomy of the periodontal pocket. Other treatment modalities include systemic anti-microbial therapy, locally placed subgingival anti-infective agents, full mouth disinfection, chlorhexidine rinsing after debridement, topical anti-microbials and intra-oral irrigation with and without chemotherapeutic agents.

Antibacterial activity of boron, which is a bioactive trace element and frequently found in diets plentiful in foods such as fruits, vegetables, and nuts, has been reported to have a regulatory effect in the inflammatory and immune response [4,5]. A boron-containing compound (AN0128) was also recently reported to have both antibacterial and anti-inflammatory properties [6]. It has been shown to reduce the formation of inflammatory infiltrate and bone loss in rats measured histologically and by micro-computerized tomography. Furthermore, it has been noted that it has *in-vitro* activity against some bacteria associated with periodontal disease, namely, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Eubacterium nodatum*, and

Treponema denticola. It has been stated that boric acid could be an alternative to chlorhexidine, and it might be more favorable because boric acid was superior in reducing the bleeding on probing, pocket depth and clinical attachment loss reductions for moderate pockets in the early healing phase [7]. The effects of 0.75% boric acid gel were recently evaluated when delivered subgingivally and was shown to promote bone formation in intrabony defect sites, thereby reducing PPD and clinical attachment level [8]. Similar improvement was seen in class II furcation defects where 0.75% boric acid gel was placed and a significant percentage of bone fill was observed [9].

In light of the above-stated evidence, the present study was designed to determine the effect of subgingival 0.75% Boric Acid (BA) irrigation as an adjunct to Non-Surgical Periodontal Therapy (NSPT) compared with 0.2% Chlorhexidine (CHX) and saline solutions on clinical and microbiological parameters in patients with chronic gingivitis.

Materials and Methods

Source of data

In this longitudinal, interventional study, 60 systemically healthy patients presenting to the Department of Periodontology, The Oxford Dental College, Bangalore, Karnataka, India fulfilled the selection criteria and recruited for the study. The entire nature and scope of the study were explained in detail to all subjects participating in the study and informed consent was obtained. The study was conducted according to the 1975 Helsinki Declaration, as revised in 2000, and was approved by the institutional ethics committee of the Oxford Dental College, Bangalore (synopsis no.: 02_D026_63558).

Selection criteria

Patients included in the study were selected based on the inclusion criteria: (a) age group of 18-40 years who were systemically healthy individuals, (b) presence of minimum of 20 teeth, (c) presence of moderate to severe gingivitis {Plaque Score (PI) score ≤ 3, Gingival Index (GI) score ≤ 3, Sulcular Bleeding Index (SBI) ≤ 5 and Probing Pocket Depth (PPD) ≤ 3mm}, (d) no history of periodontal therapy or antibiotic or anti-inflammatory therapy in the past 12 months, (e) history of compliance with oral hygiene instructions and periodic recall and (f) radiographic analysis should show absence of alveolar bone loss.

Patients were excluded if they presented with systemic disorders and platelet disorders, have used any mouth rinse within the last 3 months, smokers, pregnant or lactating mothers and any use of hormone contraceptives.

Patients satisfying the selection criteria were assigned *via* computer-generated software, into three treatment groups: I. SRP+ saline irrigation, II. SRP+ 0.2% CHX irrigation, III. SRP+ 0.75% BA irrigation. All clinical parameters pre- and post- treatment were recorded by a single clinician who also provided treatment to all the groups. Patients were masked for allocation into the treatment groups. The clinical parameters assessed at baseline, 2 weeks and one month following SRP included PI [10], GI [11], SBI [12], and PPD. William's periodontal probe was used to standardize the measurement of the clinical parameters.

Primary and secondary outcome measures

The primary outcomes of the study included the PI, GI and SBI score, while the secondary outcome included PPD and bacterial counts difference.

Formulation of 0.75% boric acid irrigant solution

Based on the *in-vitro* cytotoxicity experiments performed in a previous study, 0.75% concentration of BA solution was decided to be used for subgingival irrigation [7]. The formulation was prepared similar to the study by Saglam et al., by dissolving the weighed amount of BA in distilled water.

Clinical treatment

SRP was performed thoroughly using an ultrasonic scaler at baseline, following the collection of subgingival plaque. Subgingival irrigation was performed immediately after SRP with 10 ml of experimental irrigant (saline solution, 0.2% CHX or 0.75% BA) for 1 min at selected sites using a blunted needle and syringe. After irrigation, the patients received oral hygiene instructions which were reinforced at each revisit.

Plaque sample collection

Prior to SRP and subgingival irrigation, subgingival pooled plaque samples were Atraumatically collected at baseline, and following SRP at 2 weeks and one-month. This was performed by inserting a sterile Gracey curette using a gentle pull-stroke into the selected subgingival crevice which was properly isolated with cotton and gently dried with compressed air to prevent contamination from saliva. The plaque sample was transferred immediately into Reduced Transport Fluid (RTF).

Microbial analysis

After collection of the plaque sample in the RTF solution, it was vortexed and then diluted to 1:10 proportion. Following which, it was inoculated in the culture medium. The medium chosen for the cultivation of *P. gingivalis* and *P. Intermedia* was blood agar which consisted of Brucella agar with hemin and vitamin K. The selectivity for *P. intermedia* was enhanced by the addition of kanamycin and vancomycin. A similar methodology was followed in earlier studies by Pfau et al. and Nakayama K. [13,14]. Following the inoculation, the blood agar was incubated at 37 ˚C for 3-4 days in a strictly anaerobic environment provided by an anaerobe jar. Once the incubation was completed, the plates were removed and the colony characters were identified by gram staining technique and quantified by counting of the colony forming units.

Statistical analysis

To achieve 95% power of the study, 20 patients were recruited into each treatment group and this sample size was calculated based on data from previous studies [7]. Data were statistically analyzed using SPSS version 20.0 (IBM, Chicago, IL, USA). The following statistical tools were used to analyze the data: mean and confidence interval. One-way ANOVA was done for the comparison between the three groups. While paired t-test was done to statistically compare the parameters in each group separately. A *p-value* of <0.05 was considered to be statistically significant.

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Table 1: Comparison of whole-mouth clinical parameters at baseline, two weeks and one-month (mean ± SD).

Parameters	Baseline	Two- weeks	p- value*	One-month	p- value*
PI					
Saline	1.90 ± 0.26	1.13 ± 0.31	<0.001	1.30 ± 0.35	<0.001
Chlorhexidine	1.93 ± 0.43	1.08 ± 0.48	<0.001	1.01 ± 0.42	<0.001
Boric acid	1.73 ± 0.38	1.09 ± 0.30	<0.001	1.26 ± 0.33	<0.001
p-value**	0.187	0.386		0.006	
GI					
Saline	1.72 ± 0.33	1.00 ± 0.28	<0.001	0.98 ± 0.24	<0.001
Chlorhexidine	1.78 ± 0.39	1.10 ± 0.40	<0.001	1.03 ± 0.43	<0.001
Boric acid	1.60 ± 0.34	1.16 ± 0.35	<0.001	1.09 ± 0.29	<0.001
p- value**	0.293	0.356		0.47	
SBI					
Saline	2.19 ± 0.86	1.00 ± 0.57	<0.001	0.96 ± 0.48	<0.001
Chlorhexidine	2.28 ± 0.99	0.86 ± 0.58	<0.001	0.62 ± 0.46	<0.001
Boric acid	1.98 ± 0.74	0.55 ± 0.31	<0.001	0.57 ± 0.28	<0.001
p-value**	0.541	0.007		0.013	

*p-value refers to statistically significant difference for each group compared to baseline.
**p-value refers to statistically significant difference between groups in the same period.

Table 2: Difference in PPD.

Parameter	Baseline	Two-weeks	Difference (0-2 weeks)	p-value*	One-month	Difference (0-1 month)	p-value*
PPD							
Saline	2.82 ± 1.00	2.61 ± 1.22	0.20 ± 0.33	0.013	2.52 ± 1.15	0.29 ± 0.29	<0.001
Chlorhexidine	2.40 ± 1.27	2.10 ± 1.06	0.29 ± 0.38	0.003	1.89 ± 0.87	0.51 ± 0.58	0.001
Boric acid	1.96 ± 0.43	1.64 ± 0.50	0.31 ± 0.31	<0.001	1.59 ± 0.49	0.37 ± 0.31	<0.001
p-value**	0.005	0.006			0.008		

*p-value refers to statistically significant difference for each group compared to baseline.
**p-value refers to statistically significant difference between groups in the same period.

Results

At the end of the one-month trial period, no adverse reactions to the experimental irrigant solutions were reported from the 60 patients who completed the study. A significant reduction in clinical parameters in all the treatment groups from baseline to one-month was noted, but on the comparison between the groups, no statistically significant difference was found for levels of GI, SBI and PPD (Table 1 and 2). However, a statistically significant reduction was observed in PI for 0.2% CHX group, when compared to the 0.75% BA group and saline group (p = 0.006) (Table 1).

The mean levels of *P. gingivalis* and *P. intermedia* have shown a statistically significant reduction in all the treatment groups. On comparison of the mean reduction levels of *P. gingivalis* between the three groups, it was observed that 0.75% BA group had the greatest reduction which was statistically significant (p = 0.023). The same was, however, not observed for the mean level reduction in *P. intermedia* which was statistically insignificant for the three groups (Table 3).

Discussion

In this study, the additive effects of 0.75% BA as an alternative adjunct to 0.2% CHX and saline sub-gingival irrigation were compared on the basis of clinical and microbiological parameters in patients with gingivitis. The levels of two common periodontal pathogens, i.e. *P. gingivalis* and *P. intermedia* were assessed to determine the efficacy of the irrigants at the end of two weeks and one month.

The mean levels of the clinical parameters: GI, SBI, and PPD showed a significant decrease in all the treatment groups. This improvement in the gingival inflammatory condition shows the potent anti-inflammatory action of BA, which is in accordance with the studies by Kanoriya et al. and Singhal et al. [8,9]. However, no statistically significant differences were observed between the three groups. Braatz et al. and Watts et al. had found similar results, where they concluded that adjunctive irrigation with CHX did not provide any additional clinical benefit when compared with conventional treatment [15,16].

In the present study, the mean difference of PI values between the three groups at the end of one-month revealed that 0.2% CHX group had the highest value of 0.9175 and 0.75% BA group had the least value of 0.4705 and this difference was statistically significant (p value=0.006). These results were similar to other research by Walsh

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Table 3: Bacterial counts (CFU to be multiplied by 1000 to obtain CFU/ml).

Bacteria	Baseline	Two-weeks	p-value*	One-month	p-value*
Pg					
Saline	71.25 ± 43.40	16.95 ± 17.56	<0.001	40.1 ± 36.92	0.017
Chlorhexidine	83.25 ± 51.12	6.1 ± 7.97	<0.001	11 ± 13.87	<0.001
Boric acid	82.25 ± 64.9	4.7 ± 5.93	<0.001	4.4. ± 4.5	<0.001
p-value**	0.738	0.003		<0.001	
Pi					
Saline	93.75 ± 64.09	7.7 ± 6.95	<0.001	17.15 ± 19.29	<0.001
Chlorhexidine	72.75 ± 52.32	0.7 ± 1.41	<0.001	8.25 ± 18.03	<0.001
Boric acid	58 ± 65.03	4.25 ± 7.43	0.001	6.7 ± 17.60	<0.001
p- value**	0.183	<0.001		0.16	

*p-value refers to statistically significant difference for each group compared to baseline.
**p-value refers to statistically significant difference between groups in the same period.

et al and Mohammadi and Abbot [17,18]. The superior effect of CHX can be attributed to its substantive nature which helps maintain a potent sustained release and ability to help inhibit adherence of microorganisms to a surface thereby, preventing growth and development of biofilms [17,18].

The mean microbial levels of *P. gingivalis* and *P. intermedia* decreased significantly in all the groups after treatment. The reduction in the mean levels of *P. gingivalis* between baseline and one-month were statistically significantly higher in 0.75% BA group when compared to saline group and 0.2% CHX group (p = 0.023). This reduction in P. gingivalis levels can be attributed to the anti-bacterial and anti-inflammatory effects of boric acid as mentioned by Luan et al. who reported that boron-containing compound AN0128 showed activity againstsome bacteria associated with periodontal disease, i.e *P. intermedia, P. gingivalis, E. nodatum* and *T. denticola* with minimum inhibitory concentrations of <0.5 mg/mL [6]. The superiority of BA to CHX can be explained by the decreased anti-bacterial effect of CHX when exposed to serum proteins in the GCF [19]. Grenier et al. reported that *P. gingivalis* releases vesicles that bind to and inactivate chlorhexidine, thus protecting themselves from that agent. Due to its unique physicochemical characteristics, anti-inflammatory properties and minimally genotoxic effect on bacteria, boron has gained significant attention in the medical area [20].

The reduction in *P. intermedia* was statistically not significant (p> 0.05). This result is in accordance with the study by Saglam et al. who reported no significant differences between 0.75% BA and 0.2% CHX subgingival irrigation in terms of reduction of perio pathogens [7].

The concentration of BA was safely used based on the results of *in-vitro* cytotoxicity experiments conducted by Saglam et al. it was decided to use 0.75% concentration of BA (pH= 4.9) in this clinical study. Only three higher concentrations of BA (6%, 3%, and 1.5%) reduced cell survival of human periodontal ligament fibroblasts and human gingival fibroblasts significantly [7]. This concentration was similarly used in gel form in previous studies without any adverse reactions [8,9]. In contrast, Alleyn et al. demonstrated the exposure of root surfaces to 0.12% CHX which significantly inhibited subsequent fibroblast attachment, therefore impeding regeneration of the periodontium. CHX was found to be highly cytotoxic for human periodontal ligament cells by inhibiting double-stranded nucleic acid content, protein synthesis, and mitochondrial activity [21]. In a study by Pucher and Daniel, CHX was demonstrated to be cytotoxic for human fibroblasts via inhibition of protein synthesis [22]. In the present study, no additional effect of 0.2% CHX to conventional treatment for PPD in the early healing period was observed. This might be a result of the cytotoxic and inhibitory effect of CHX on the fibroblasts. In a study by Arabaci et al. in 2013, the results showed a dose-dependent genotoxic and cytotoxic effect of CHX on human lymphocytes in vitro and therefore, concluded that periodontal irrigation should be done with lower concentrations of CHX [23].

As reported by Ince et al., boric acid prevents oxidative damage by increasing an antioxidant agent, glutathione, and its analog and by promoting other neutralizing agents of reactive oxygen species [24]. This may be the reason why significant reduction in PPD was observed in 0.75% BA group.

This present study was the first study conducted on patients suffering from gingivitis. The follow-up period was only of one-month duration and this was one of the limitations of the study. Studies with a longer duration may be required to evaluate the longevity of the effect of the irrigants. Another important shortcoming of the study is the method used to detect the periodontal pathogens. The bacterial culture method with an accuracy of 61-79% is considered to be least reliable [25]. Hence, more accurate methods, such as DNA probes could enhance the results of this study.

Conclusion

Within the limits of the study, the present investigation shows that the use of 0.75% BA or 0.2% CHX as an adjunct to NSPT did not reveal any statistically significant differences in clinical parameters compared with conventional treatment. The adjunctive 0.75% BA irrigation, however, produced beneficial improvement in the levels of P. gingivalis. Hence, SRP with 0.75% BA irrigation can be considered as an adjunctive approach in the treatment of moderate to severe gingivitis. Longitudinal, multicenter, randomized, controlled clinical trials are, however, required to confirm the findings of the present study.

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