



Comparative Evaluation of Tongue Coat Inhibitory Effect of 1% Stabilized Chlorine Dioxide Mouthrinse with that of 0.2% Chlorhexidine Gluconate Mouthrinse in the Treatment of Halitosis using N-benzoyl-DL-arginine-2-naphthylamide Assay: A Randomized Crossover Study

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ABSTRACT

Aim: To evaluate the efficacy of 1% Stabilized Chlorine dioxide (ClO₂) mouthrinse as compared with 0.2% Chlorhexidine gluconate (CHX) mouthrinse on tongue coat assessed by N-benzoyl-DL-arginine-2-naphthylamide (BANA) assay in the treatment of halitosis.

Materials and methods: Twenty systemically healthy subjects with self-reported halitosis were enrolled in the study as per the inclusion criteria. The participants were assigned to use either aqueous 1% stabilized ClO₂ mouthrinse (Group I) or aqueous 0.2% CHX mouthrinse (Group II). The study was conducted in two phases of 15 days duration each with an intervening washout period of 7 days. Subjects were assessed at baseline and 15 days in each phase for oral hygiene using Plaque index (PI) and Winkel tongue coating index (WTCI). Volatile sulfur compound (VSC) in exhaled breath was measured using portable sulfide monitor. On the 15th day, tongue coating samples were assessed for both Groups using chair-side BANA assay.

Results: Both Groups I and II demonstrated significant reduction in PI scores at 15 days as compared with baseline. However, reduction in PI score was found to be statistically significant in favor of Group I as compared with Group II after 15 days (p-value: 0.001). Reduction in WTCI scores was statistically nonsignificant for both Groups I and II at 15 days (p-value: 0.094). Qualitative VSC scores by portable sulfide monitor on assessment of exhaled breath was statistically nonsignificant for both Groups I and II at 15 days (p-value: 0.131). The BANA scores for tongue coat samples from both Groups were comparable at 15 days with no statistical significance (p-value: 0.503).

Conclusion: The observation from present study settings would lead to infer that dental plaque inhibition, tongue coat inhibition, and VSC production are comparable for both the Groups in the treatment of physiological halitosis.

Keywords: Chlorhexidine, Chlorine dioxide, Halitosis, N-benzoyl-DL-arginine-2-naphthylamide, Tongue coat.

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INTRODUCTION

Halitosis or oral malodor is an offensive odor coming from the oral cavity.^{1,2} Although some extraoral systemic and metabolic conditions like respiratory tract diseases, gastrointestinal disorders, and diabetes mellitus have been associated with oral malodor, intraoral causes of oral malodor include plaque-induced diseases of gingivitis and periodontitis. Tongue coats on the dorsum of the tongue are also attributed to halitosis, particularly physiologic halitosis.^{3,4} Physiologic halitosis is commonly experienced as morning breath attributed to accumulation of tongue coat overnight in the interlude between oral hygiene routine and stagnation of saliva.⁵

Proteolytic bacteria found within the accumulated tongue coat produce VSC by protein degradation contributing to characteristic odors of physiologic halitosis. The substrates for VSC are mainly sulfur-containing amino acids that are found in saliva, gingival crevicular fluid, and acquired tongue coats.⁶ The VSCs, such as hydrogen sulfide, methyl mercaptan, and dimethyl sulfide are odorous gases commonly detected in exhaled air in subjects with physiologic halitosis. For assessment and measurement of VSCs, handheld sulfide monitors have been used and have demonstrated dependable reproducible recordings. Tongue coats have been found to be comprised of proteolytic anaerobic gram-negative bacteria, *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), and *Treponema denticola* (Td) that produce VSCs.⁷

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The Pg, Td, and Tf break down dietary and host-associated protein found in tongue coats of healthy adults on the dorsum of the tongue producing VSCs of hydrogen sulfide and methyl mercaptan leading to halitosis. The dorsum of the tongue accumulate tongue coats, as they are difficult to debride for most patients. A chairside diagnostic assay i.e. BANA assay detects the presence of these bacteria within oral plaque samples and tongue coats based on the ability of these bacteria to hydrolyze the substrate BANA leading to a colorimetric change indicative of levels of presence of afore-mentioned bacteria.⁸⁻¹¹

Oral antiseptics used as mouthrinses containing CHX, chlorine dioxide (ClO₂), essential oils, and cetylpyridinium chloride have been previously used in the treatment of halitosis;¹² 0.12% and 0.2% CHX has demonstrated good efficacy in the management of self-perceived halitosis by its established antiseptic effects. However, the prolonged use of CHX mouthrinse is associated with side effects of tooth staining, tongue staining, and dysgeusia.¹³ The ClO₂ is associated with release of nascent singlet oxygen that disrupts nutrient transport across bacterial cell membranes, thereby exerting antiseptic properties; moreover ClO₂ mouthrinse and the chlorite anion (ClO₂⁻) are attributed to direct oxidation of VSCs to nonmalodorous products.¹⁴ This study aims to compare these two mouthrinses on their tongue coat inhibitory effect to explore their therapeutic implications for the management of physiologic halitosis.

AIM

The aim of this article is to assess and evaluate the effect of aqueous 1% stabilized ClO₂ mouthrinse as compared with aqueous 0.2% CHX mouthrinse on tongue coat assessed by BANA assay in the treatment of physiologic halitosis.

OBJECTIVES

To comparatively evaluate the tongue coat inhibitory effect of aqueous 1% ClO₂ mouthrinse (Group I) and aqueous 0.2% CHX mouthrinse (Group II) in healthy volunteer subjects with self-perceived physiologic halitosis by assessment of

- Plaque index score (PI) (Silness and Loe)¹⁵ between baseline and 15 days.
- Portable sulfide monitor (Breath Alert™, Tanita Corporation, Japan) VSC level score read out between baseline and 15 days.
- Winkel tongue coating index score (WTCI) between baseline and 15 days.
- Chairside BANA test (BANA-Zyme®, Oratec, USA) score on tongue coat sample at the 15th day.

MATERIALS AND METHODS

This clinical, randomized, double-blinded, crossover study with a washout period of 7 days between the phases of the study was performed on 20 healthy volunteer subjects. The study was granted ethical clearance from the institutional ethical committee abiding to all human ethical principles as per the World Medical Association Declaration of Helsinki and the Guidelines of Good Clinical Practice of Indian Council of Medical Research being observed. The study population comprised 20 healthy volunteers (6 males, 14 females) with self-perceived physiologic halitosis between the ages of 18 and 25 years with the mean age of 20.8 years, who met the inclusion criteria of the study and rendered consent to their participation by signed document.

The inclusion criteria were:

- Subjects aged 18 years and above rendering informed consent
- Subjects with self-perceived physiologic halitosis on screening by portable sulfide monitor at baseline (VSC score >1)
- Good oral hygiene with a mean PI score < 1
- Minimum 20 natural scorable teeth excluding third molars to be present during examination
- Subjects complying with oral hygiene routine throughout the study

Exclusion criteria were:

- Subjects with signs of plaque-induced gingival or periodontal disease
- Subjects having received any surgical or nonsurgical periodontal therapy in the past 6 months
- Subjects who have been administered any antimicrobial and/or anti-inflammatory therapy within the past 1 month
- Subjects wearing orthodontic or prosthetic appliances
- Subjects who give present or past history of drug abuse
- Subjects with unrestored carious teeth
- History of allergy to the mouthrinses assigned in the study
- Subjects with mouth breathing and occlusal para function
- Smokers and tobacco chewers
- Alcohol consumption

At baseline visit, VSC levels were assessed using a portable sulfide monitor (Breath Alert™). Measurements were conducted at around 9 am on the assessment day to evaluate morning breath odor. Prior to assessment, subjects were advised to abstain from eating pungent food and from using scented cosmetics for 24 hours prior to assessment and report without performing oral hygiene routine on the morning of the

assessment. Subjects were asked to hold their breath for 5 seconds with the lips sealed and then exhale gently through the mouth toward the sensor of the portable sulfide monitor until score is registered on a scale of 0 to 5 (Fig. 1). Plaque index and WTCI were recorded following which tongue cleaning and scaling were performed and oral hygiene instructions were given for all subjects where they were advised to rinse with 10 mL of undiluted mouthrinse A or mouthrinse B for 1 minute twice a day, i.e., in the morning following toothbrushing after breakfast and at night following toothbrushing after dinner, for a period of 15 days. Rinsing was to be followed by gargling for 10 seconds. During the 15-day study period, subjects used fluoridated dentifrice for brushing twice daily, using modified bass technique with a medium bristle toothbrush with no attempts made toward mechanical tongue cleaning.

Following two mouthrinses were assigned by computer-generated randomization:

Group I: Aqueous 1% stabilized ClO₂ mouthrinse Freshchlor® (Rowpar Group Pharmaceuticals, Bengaluru, India)

Group II: Aqueous 0.2% CHX mouthrinse Hexidine® (ICPA, Bengaluru, India)

For the study to be blinded, the mouthrinses were supplied in identical white opaque bottles along with measuring caps labeled as mouthrinse A and mouthrinse B. Subjects were randomly assigned using computer-generated random numbers to either Group I or Group II. For the first experimental phase, subjects were assigned with either mouthrinse A or mouthrinse B. In the second experimental phase, after a 7-day washout period, each group then used the other mouthrinse not assigned during the first phase for 15 days, rendering both Groups with 20 subjects having used both mouthrinses over the study duration. Subjects were asked to revert to their

normal routine of oral hygiene during the washout period of 7 days following which the second experimental phase was commenced for 15 days using either of the mouthrinses A or B not used during the first phase of the study. Subjects were asked to report back for assessment after 15 days.

On the 15th day of completion of either phase of the study when subjects reported for assessment, VSC scores, PI, and WTCI were recorded (Fig. 2). Tongue coat samples through scrapings were then collected from the dorsal surface of the tongue using cotton tip swab. The chairside BANA assay was performed for the collected tongue coat samples by application of the tongue coat samples on to the raised reagent matrix affixed to the lower portion of the BANA test strip. The upper end of the BANA test strip (salmon-colored end) was moistened with distilled water and folded onto the lower reagent end of the BANA strip and introduced into the processor, activating the processor heating element. The BANA test strip was left in the processor at 55°C for a duration of 5 minutes. The BANA test strip from the processor was then removed and color change on the upper end of test strip was noted. If a blue color change from salmon was detected, then the site was marked as either weak positive or positive, depending on the intensity of the color change as per color guide provided with the kit. Recording was done for each sampled site as negative, weak positive, or positive (0 to 2) (Fig. 3).

The detailed flowchart of the study is depicted in Flow Chart 1.

Statistical Analysis

The data were tabulated and submitted to a blinded statistician and analyzed using Statistical Package for the Social Sciences software version 17.0. Based on data



Fig. 1: Assessment of VSC levels using portable sulfide monitor (Breath Alert™)

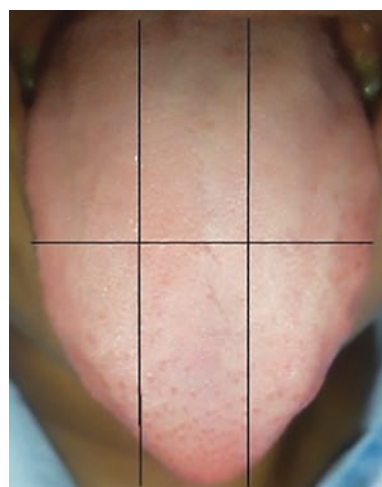


Fig. 2: Assessment of WTCI

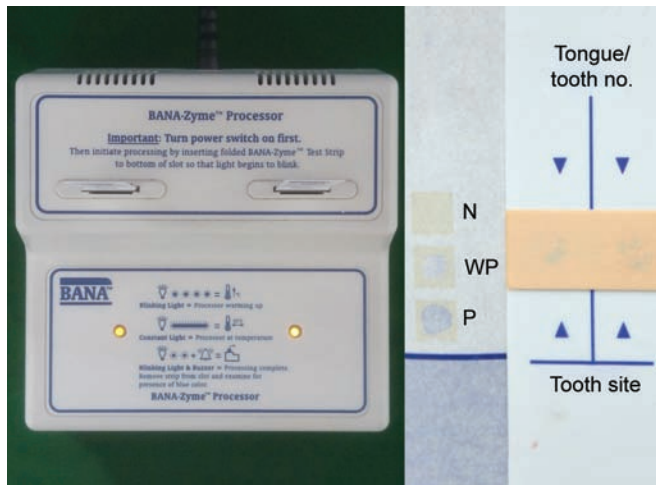


Fig. 3: Assessment of BANA score using chairside BANA-Zyme assay

for both Groups I and II, it was found that both Groups I and II demonstrated significant reduction in PI, VSC, and WTCI scores (p -value < 0.05) (Table 1). As baseline data for both the Groups were not normally distributed, the difference in mean values at baseline and 15 days was used for intergroup comparison which demonstrated significant reduction in PI score in favor of Group I as compared with Group II at 15 days (p -value = 0.001). Reduction in VSC scores was statistically nonsignificant between Groups I and II at 15 days (p -value = 0.131). Reduction in WTCI score was statistically nonsignificant between Groups I and II at 15 days (p -value = 0.094) (Table 2). Intergroup comparison of BANA scores at 15 days was statistically nonsignificant between Groups I and II (p -value = 0.503) (Table 3).

analysis, paired t-test was used for intragroup comparison and independent t-test was used for the intergroup comparison with a p -value < 0.05 treated as statistically significant.

RESULTS

Test of normality was applied using Kolmogorov-Smirnov test and Shapiro-Wilk test, revealing that data for both groups at baseline were not normally distributed. In intragroup comparison between baseline and 15 days

DISCUSSION

The present study comparatively evaluated the tongue coat inhibitory efficacy of aqueous 1% stabilized ClO_2 mouthrinse with that of aqueous 0.2% CHX mouthrinse by using WTCI and BANA assay in subjects under treatment for self-perceived physiologic halitosis over a period of 15 days. The quality of exhaled air from the mouth of these subjects was measured using a portable sulfide monitor through assessment of VSC levels at baseline and 15 days. Tongue coat inhibition was measured

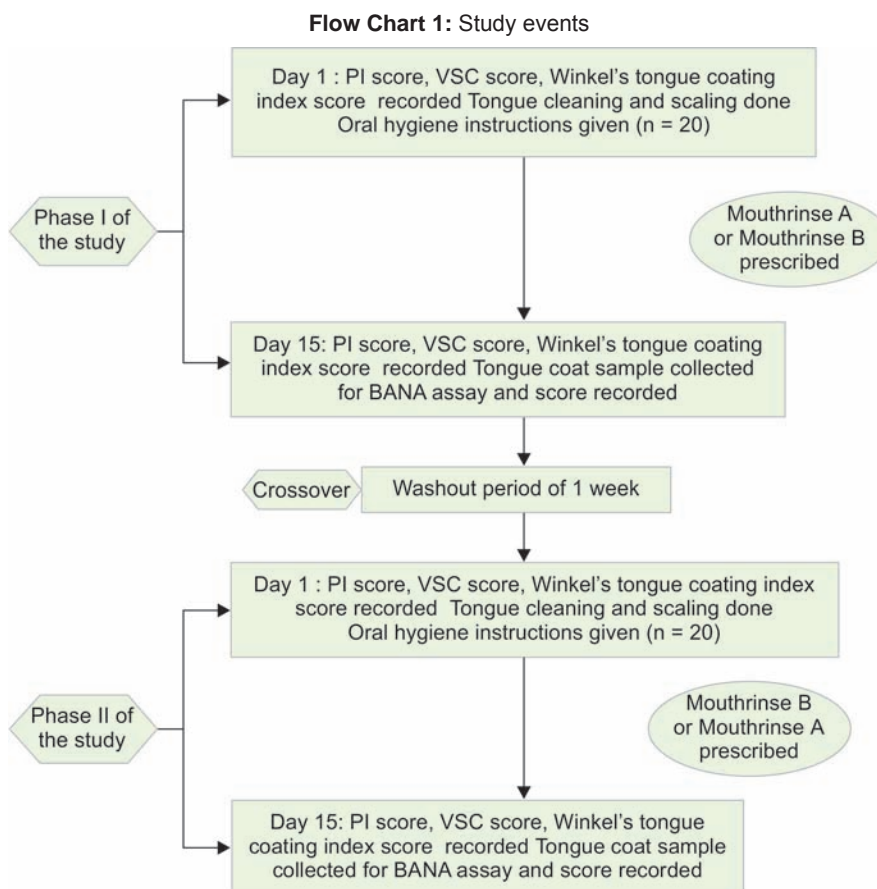


Table 1: Intragroup comparison of mean values of PI, VSC, and WTCI between baseline and 15 days for groups I and II

Parameter	Group	Baseline (mean \pm SD)	15 days (mean \pm SD)	p-value
PI	I	0.36 \pm 0.01	0.34 \pm 0.01	<0.001*
	II	0.38 \pm 0.01	0.35 \pm 0.01	<0.001*
VSC	I	2.55 \pm 0.51	1.10 \pm 0.72	<0.001*
	II	3.30 \pm 0.57	1.45 \pm 0.51	<0.001*
WTCI	I	5.10 \pm 0.79	4.30 \pm 0.47	<0.001*
	II	5.70 \pm 0.80	4.55 \pm 0.60	<0.001*

SD: Standard deviation; *p-value <0.05

Table 2: Intergroup comparison of differences of mean values of PI, VSC, and WTCI between baseline and 15 days for groups I and II

Parameters	Group I (mean \pm SD)	Group II (mean \pm SD)	Mean difference	t-test	p-value
PI score	0.01 \pm 0.007	0.02 \pm 0.124	0.01225	3.803	0.001*
VSC score	1.45 \pm 0.825	1.85 \pm 0.812	0.4	1.544	0.131
WTCI score	0.80 \pm 0.615	1.15 \pm 0.670	0.35	1.719	0.094

SD: Standard deviation; *p-value <0.05

Table 3: Intergroup comparison of mean values of BANA score at 15 days between groups I and II

Parameters	Group I (mean \pm SD)	Group II (mean \pm SD)	Mean difference	t-test	p-value
BANA score	0.25 \pm 0.444	0.35 \pm 0.489	0.1	0.677	0.503

SD: Standard deviation

quantitatively by difference in WTCI at 15 days from baseline, and qualitatively by chairside BANA assay of tongue scrapings at 15 days. The present study was a randomized, double-blinded crossover clinical study with an intervening 1 week washout period. Each subject was given assigned respective mouthrinse during each phase of the crossover study by computer-generated randomized sequence method. Therefore, upon conclusion of the study, all subjects had been assigned to both mouthrinses to minimize possible interindividual variability.

In the present study, both ClO₂ mouthrinse and CHX mouthrinse reduced PI scores at 15 days as compared with baseline. However, reduction in PI score was statistically significant in favor of ClO₂ as compared with CHX based on difference in mean PI scores between baseline and 15 days (p-value<0.05). This observation was not in agreement with Yadav et al¹⁶ where plaque score reductions were found to be comparable between ClO₂ and CHX in a 4-day plaque model study or with Paraskevas et al¹⁷ in 2008 where during a 3-day *de novo* plaque accumulation model, plaque score reduction with CHX was found to be superior as compared with ClO₂.

The VSCs are released from the breakdown of proteins from tongue coats by Pg, Td, and Tf bacteria contributing to oral malodor. Both ClO₂ and CHX mouthrinse demonstrated reduction in VSC scores at 15 days compared with baseline (p-value<0.05). This observation was in agreement with Rosenberg et al¹⁸ who observed reduction in VSC levels after usage of 0.2% CHX solution, and Shinada

et al¹⁹ who observed that ClO₂ mouthrinse was efficacious in reducing morning oral malodor when used for a 7-day period. Similarly, Peruzzo et al²⁰ found that ClO₂ can maintain VSCs at lower levels in the morning breath after assessment in a 4-day plaque model. However, on intergroup comparison, it was found that there was no statistical difference in reduction of VSC level at 15 days from baseline between ClO₂ and CHX mouthrinse (p-value = 0.131).

Reduction in WTCI scores was statistically significant for both ClO₂ and CHX used as a rinse after 15 days compared with baseline (p-value <0.05). This was in agreement with Hakuta et al's²¹ finding that mechanical effect of mouthrinsing and gargling led to reduction in tongue coating. However, on intergroup comparison, it was found that there was no statistical difference in reduction of WTCI level at 15 days from baseline between ClO₂ and CHX mouthrinse (p-value=0.094).

The chairside BANA assay used in this study, which detects the presence of Pg, Td, and Tf bacteria based on colorimetric change on ability to hydrolyze the synthetic trypsin substrate, revealed no difference between ClO₂ and CHX on assessment of tongue coat scrapings at 15 days. De Boever and Loesche¹⁰ found that individuals with halitosis demonstrated tongue coating samples with positive scores for BANA with high organoleptic scores correlating to positive BANA scores. However, to the best of our knowledge, while conducting the study, we did not come across any literature comparing ClO₂ and CHX in direct correlation with BANA scores.

CONCLUSION

Within the limitations of the study, the inference derived from present study settings would be that dental plaque inhibition, tongue coat inhibition, and VSC level reduction are comparable for subjects using either aqueous 1% ClO₂ or aqueous 0.2% CHX in the treatment of physiologic halitosis.

LIMITATIONS

Anticipated Hawthorne effect could possibly have contributed to good plaque control in both groups as the study period was relatively small; however, the crossover study design was aimed at minimizing this effect. Halitosis was assessed mainly objectively through VSC assessment by a portable sulfide monitor; however, the self-perception by organoleptic assessment by subjects themselves or odor judges could have added further value to the study.

REFERENCES

- Krespi YP, Shrimme MG, Kacker A. The relationship between oral malodor and volatile sulfur compound-producing bacteria. *Otolaryngol Head Neck Surg* 2006 Nov;135(5):671-676.
- Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. *J Periodontol* 1977 Jan;48(1):13-20.
- Miyazaki H, Sakao S, Katoh Y, Takehara T. Correlation between volatile sulfur compounds and certain oral health measurements in the general population. *J Periodontol* 1995 Aug;66(8):679-684.
- Murata T, Yamaga T, Iida T, Miyazaki H, Yaegaki K. Classification and examination of halitosis. *Int Dent J* 2002 Jun;52 (Suppl 3):181-186.
- Ohmori M, Imai R, Satoh S, Hori R, Hasegawa A. A study of daily in physiological oral malodor. *J Jpn Soc Periodontol* 1999;42:43-48.
- Yaegaki K, Sanada K. Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease. *J Periodontol Res* 1992 Jul;27(4 Pt 1):233-238.
- Loesche WJ, Kazor C. Microbiology and treatment of halitosis. *Periodontol* 2000 2002;28:256-279.
- Loesche WJ, Bretz WA, Kerschensteiner D, Stoll J, Socransky SS, Hujuel P, Lopatin DE. Development of a diagnostic test for anaerobic periodontal infections based on plaque hydrolysis of benzoyl-DL-arginine-naphthylamide. *J Clin Microbiol* 1990 Jul;28(7):1551-1559.
- Kozlovsky A, Gordon D, Gelernter I, Loesche WJ, Rosenberg M. Correlation between the BANA test and oral malodor parameters. *J Dent Res* 1994 May;73(5):1036-1042.
- De Boever EH, Loesche WJ. Assessing the contribution of anaerobic microflora of the tongue to oral malodor. *J Am Dent Assoc* 1995 Oct;126(10):1384-1393.
- Jose AA, Feres M, Figueiredo LC, Salvador SL, Cortelli SC. The ability of the BANA test to detect different levels of *P. gingivalis*, *T. denticola* and *T. forsythia*. *Braz Oral Res* 2010 Apr-Jun;24(2):224-230.
- Winkel EG, Roldán S, Van Winkelhoff AJ, Herrera D, Sanz M. Clinical effects of a new mouthrinse containing chlorhexidine, cetylpyridinium chloride and zinc-lactate on oral halitosis. A dual-center, double-blind placebo-controlled study. *J Clin Periodontol* 2003 Apr;30(4):300-306.
- Gürkan CA, Zaim E, Bakirsoy I, Soykan E. Short-term side effects of 0.2% alcohol-free chlorhexidine mouthrinse used as adjunct to non-surgical periodontal treatment: a double blind clinical study. *J Periodontol* 2006 Mar;77(3):370-384.
- Noszticzus Z, Wittmann M, Kály-Kullai K, Beregvári Z, Kiss I, Rosivall L, Szegedi J. Chlorine dioxide is a size-selective antimicrobial agent. *PLoS One* 2013 Nov 5;8(11):e79157.
- Löe H. The gingival index, the plaque index and the retention index systems. *J Periodontol Silness and Loe* Nov-Dec;38(6): Suppl:610-616.
- Yadav SR, Kini VV, Padhye A. Inhibition of tongue coat and dental plaque formation by stabilized chlorine dioxide vs chlorhexidine mouthrinse: a randomized, triple blinded study. *J Clin Diagn Res* 2015 Sep;9(9):ZC69-ZC74.
- Paraskevas S, Rosema NA, Versteeg P, Van der Velden U, Van der Weijden GA. Chlorine dioxide and chlorhexidine mouthrinses compared in a 3-day plaque accumulation model. *J Clin Periodontol* 2008 Aug;79(8):1395-1400.
- Rosenberg M, Kulkarni GV, Bosy A, McCulloch CA. Reproducibility and sensitivity of oral malodor measurements with a portable sulfide monitor. *J Dent Res* 1991 Nov;70(11): 1436-1440.
- Shinada K, Ueno M, Konishi C, Takehara S, Yokoyama S, Kawaguchi Y. A randomized, double blind, crossover, placebo-controlled clinical trial to assess the effects of a mouthwash containing chlorine dioxide on oral malodor. *Trials* 2008 Dec 9;9:71.
- Peruzzo DC, Jandiroba PF, Nogueira Filho Gda R. Use of 0.1% chlorine dioxide to inhibit the formation of morning volatile sulphur compounds (VSC). *Braz Oral Res* 2007 Jan-Mar;21(1):70-74.
- Hakuta C, Mori C, Ueno M, Shinada K, Kawaguchi Y. Evaluation of an oral function promotion program for the independent elderly in Japan. *Gerodontology* 2009 Dec;26(4):250-258.