

Clinical and Microbiological Evaluation of Chlorine Dioxide Based Mouthwash and Toothpaste in Periodontitis Patients along with Combination of Nutritional Dietary Supplement of CoQ10

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ABSTRACT

Aim: The present study to assess and compare the clinical and antimicrobial effects of sodium chlorite based toothpaste and mouthwash in periodontitis patients along with combination of nutritional dietary supplement of CoQ10 with conventional based toothpaste and mouthwash without sodium chlorite.

Materials and methods: A total 100 generalized chronic periodontitis patient between the ages of 18 and 55 years were enrolled in the study and divided under four categories. Clinical and microbiological parameters were recorded prior to phase 1 therapy; and subjects were put on different oral hygiene regime with chlorine dioxide based and conventional dentifrice and mouthrinse. CoQ10 was also provided in both the groups as nutritional dietary supplement. At 2nd and 4th month post-phase 1 therapy subjects were re-evaluated.

Results: The results of this study showed that there was significant decrease in clinical, and microbiological parameters from baseline to 4 months in both the groups ($p < 0.01$). The subjects under groups using sodium chlorite based toothpaste and mouthwash with dietary supplement of CoQ10 showed a highly significant reduction to all the parameters as compared to subjects under groups using a conventional dentifrice and mouthrinse.

Conclusion: Thus, we can conclude that long-term regular use of chlorine dioxide based products along with nutritional supplement of CoQ10 is more beneficial than conventional toothpaste and mouthrinse.

Keywords: Chlorine dioxide, CoQ10, Antioxidant.

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INTRODUCTION

The mouth acts as a window to lot of systemic diseases and serves as a port of entry to the various infections that can alter and affect the immune status of the person. The oral cavity has the potential to harbor at least 600 different bacterial species, and in any given patient, more than 150 species may be present, surfaces of teeth can have as much as billion bacteria in its attached bacterial plaque.¹ Periodontitis has been proposed as having an etiological or modulating role in cardiovascular, cerebrovascular disease,

diabetes, respiratory disease and adverse pregnancy outcome; several mechanisms have been proposed to explain or support such theories. One of these is based around the potential for the inflammatory phenomenon of periodontitis to have effects by the systemic dissemination of locally produced mediators such as C-reactive protein (CRP), interleukins -1 beta (IL-1 β) and -6 (IL-6) and tumor necrosis factor alpha (TNF- α).² Periodontal diseases are recognized as infectious processes that require bacterial presence and a host response which are further affected and modified by other local, environmental, and genetic factors. The oral cavity works as a continuous source of infectious agents, and its condition often reflects the progression of systemic pathologies.³ Dental plaque biofilm cannot be eliminated. However, the pathogenic nature of the dental plaque biofilm can be reduced by reducing the bioburden and maintaining a normal flora with appropriate oral hygiene methods.⁴ The toothbrush plays an important role for personal oral hygiene and effective plaque removal.⁵ The primary purpose of brushing the teeth with a dentifrice is to clean the accessible tooth surfaces so as to minimize the accumulation of dental plaque, stains and food debris. The recent past has witnessed resurgence in the use of sodium chlorite based dentifrices; the main application of sodium chlorite is the generation of chlorine dioxide. Likewise systemic health, diet and nutrition also impact on oral health, in particular gingival and periodontal diseases. A person's diet can exert a topical or a systemic effect on the body and its tissues. In many disease conditions connected with increased generation and the action of reactive oxygen species (ROS), the concentration of coenzyme Q10 in the human body decreases⁶⁻⁸ and the deficiency of coenzyme Q10 leads to the dysfunction of the respiratory chain, which is due to the insufficient production of highly energetic compounds, which decrease the efficiency of cells. Hence, an attempt has been made in the present study to assess and compare the clinical and antimicrobial effects of sodium chlorite based toothpaste and mouthwash in periodontitis patients along with combination of nutritional dietary supplement of CoQ10 with conventional based toothpaste and mouthwash without sodium chlorite.

MATERIALS AND METHODS

The present study was conducted in the Department of Periodontology, Rural Dental College, Loni in co-ordination with Department of Microbiology, Rural Medical College and Central Collection Laboratory (CCL) of Pravara Institute of Medical Sciences, Loni, Ahmednagar, Maharashtra, India. This clinical study was approved from the research and ethical committee of the Pravara Institute of Medical Sciences University, Loni, Ahmednagar, Maharashtra, India.

Study Population

The subjects enrolled in this study were selected from the Outpatient Department of Periodontology, Rural Dental College and Hospital, Loni. After an informed consent, a total 100 generalized chronic periodontitis patients between the ages of 18 and 55 years were enrolled in the study and divided under four categories. Each group was comprised of 25 subjects each as illustrated in Table 1. Exclusion criteria for the patient enrolled in the study were: (1) presence of any systemic neurological disorder (e.g. epilepsy or schizophrenia), (2) presence of a disease with possible effects on the immune system (e.g. chronic infections or cancer), (3) patient who have received antibiotics or NSAIDs (like Ibuprofen) in past 9 to 11 weeks, (4) patients who have received periodontal treatment in past 6 months, (5) pregnant and lactating mother, (6) patient with artificial prosthesis, (7) patients who smokes or consumes tobacco in any form, (8) patients suffering with arthritis, (9) patient with any type of heart disease (MI, CHD, etc.), (10) female patient using intrauterine birth control devices or birth control pills, (11) obese Individuals (30 and above range as per WHO BMI cut off for weight categories for Asians), (12) presence of diabetes mellitus, (13) participants not willing to participate in the study.

Clinical Protocol

Patients received a verbal description about the clinical protocol to be followed in this clinical trial. In order to have the unbiased and accurate clinical data, we followed a double blind protocol in the study for enrollment of the patients in

terms of treatment plan (Phase 1 Therapy). Also categorization of patients were done randomly, with oral products regime (With and without chlorine dioxide) to be followed after the phase 1 therapy. After enrollment of the subjects in the study, Phase 1 therapy (Complete scaling and root planing) was done by similar EMS ultrasonic scaler to all the subjects enrolled in the study. Subjects were advised to brush twice daily 5 minutes with modified bass method technique (Technique demonstrated to each subject) and similar medium bristle tooth brushes were provided to each of the enrolled subject during the study course to maintain standardization. The subjects were further advised for a mouthrinse twice daily (5 ml in quantity for 1 minute). Subjects with respective groups were further provided with dietary supplement of CoQ10 with instructions for the regular consumption for the same with regime (TDS × 4 months).

Clinical Parameters Evaluated

Clinical parameters of periodontal disease that were evaluated in all the four groups were gingival index (GI) (Loe and Silness), plaque index (PI) (Turesky-Gilmore-Glickman modification of Quigley hein plaque index), probing depth (PD) and clinical attachment level (CAL). All the measurements were done by the single operator with Williams periodontal probe at base level, 2nd and 4th month for all the groups.

Microbiological Parameters Evaluated

Subgingival plaque samples were collected for specific bacterial examination, i.e. *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Prevotella intermedia*. Subgingival plaque samples were collected from the sample sites using the standardized paper point (Dentsply®) which were inserted to the depth of the periodontal pocket until resistance was felt. The paper points were retained for 20 seconds in the collection sites. The samples site selected was maxillary first molar in all the cases to maintain the standard protocol. After 20 seconds the paper point was removed from the sample site and immediately placed into the Robertson's cooked meat

Table 1: Study groups: categorization of chronic periodontitis patients and study protocol

Groups	Scientific protocol
Group A	Chronic periodontitis patients with complete oral prophylaxis (Scaling and root planing) followed by use of chlorine dioxide based toothpaste and mouthwash. (Oxyfresh dentifrice and mouthrinse).
Group B	Chronic periodontitis patients with complete oral prophylaxis (Scaling and root planing) followed by use of conventional toothpaste and mouthwash (Without chlorine dioxide: Pepsodent dentifrice and listerine mouthrinse).
Group C	Chronic periodontitis patients with complete oral prophylaxis (Scaling and root planing) followed by use of chlorine dioxide based toothpaste and mouthwash along with dietary supplement of CoQ10 (TDS x 4 months). (Oxyfresh CoQ10 nutritional supplement).
Group D	Chronic periodontitis patients with complete oral prophylaxis (Scaling and root planing) followed by use of conventional toothpaste and mouthwash (Without chlorine dioxide) along with dietary supplement of CoQ10 (TDS x 4 months).

transport (RCM) in a test tube. The collected samples were then transferred to microbiological laboratory for specific bacterial culturing. In the laboratory the Robertson’s cooked meat medium (RCM) was subjected to vortex homogenization for 60 seconds before incubated anaerobically (Gas pack system) for 2 to 3 days. Standard loop which can hold 0.01 mm of Robertson’s cooked meat medium (RCM) was used and spread on to the culture plates and colony forming units (CFU) were counted using colony counter.

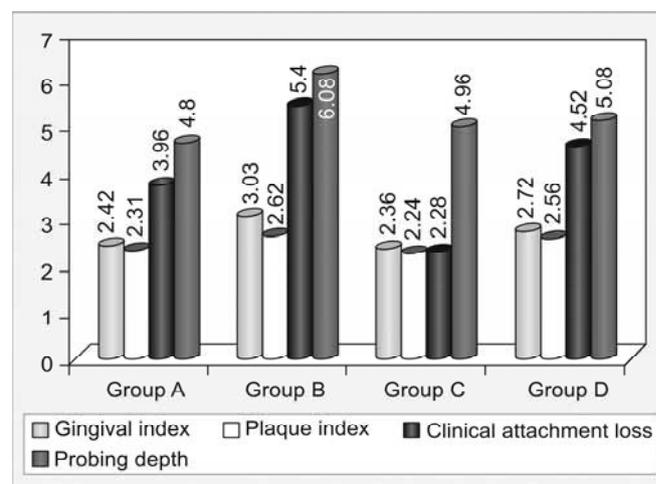
Base line clinical measurements and microbiological samples were recorded of all the four groups. Phase 1 Therapy (Full mouth scaling and root planing) was carried out in patients belonging to all the groups, i.e. groups A to D. Recall visits was scheduled for all the subjects belonging to all the groups (A to D) on 2nd and 4th month. (Both clinical and microbiological measurements were recorded). Comparative assessment was done among all the patients that were divided into with student paired ‘t’ test and ANOVA.

RESULTS

Clinical Observations

Distribution of mean and standard deviation values of clinical parameters of all the groups were illustrated in Table 2. The clinical parameters (Gingival index, plaque index and clinical attachment level) of experimental period, i.e. baseline, 2nd and 4th month were recorded and compared. Results showed a highly significant change with regard to improvement in the gingival inflammation and reduced bleeding on probing and plaque accumulation from the baseline when compared to the 2nd and 4th month. By applying Student’s unpaired ‘t’ test there was a highly significant difference between mean values of GI, PI, CAL and PD when all groups compared each other. (i.e. $p < 0.001$) as illustrated in Graph 1. Thus, it was seen that group C showed more decreased (fall) of clinical parameters than other groups in the study. That is decrease in the clinical parameters

(GI, PI, CAL and PD) was more/higher in group C followed by group A, D and B. By applying Tukey-Kramer multiple comparison test there was a highly significant difference in the mean values of gingival index, plaque index, clinical attachment loss and probing depth at 4 months in group all four groups compared together (i.e. $p < 0.0001$) where value of ‘F’ = 78.859, $p < 0.0001$, very highly significant. It was observed that group C showed highest percentage decrease of clinical parameters from base line to 4th month in the entire recordings as illustrated in Graph 2. The percentage decrease of GI, PL, CAL and PD from base line to 4th month in group C was 14.49, 15.15, 29.41 and 14.48%. This was followed by group A and the percentage decrease of GI, PL, CAL and PD from baseline to 4th month in group A was 13.26, 14.12, 19.86 and 12.94%. There was 10.22, 13.39, 17.38 and 9.79% percentage decrease of GI, PL, CAL and PD from baseline to 4th month in group D. The last with lease number of percentage decrease in clinical parameters from base line to 4th month was observed in group B with 3.015, 5.41, 4.25 and 3.78% percentage values of GI, PL, CAL and PD. It is concluded



Graph 1: Comparison of mean values of all the clinical parameters at 4 months duration in all the groups under this study

Table 2: Distribution of mean and standard deviation values (mean ± SD) of all the clinical parameters at baseline, 2nd and 4th month

Groups	Observation	GI	PI	CAL
A	Base line	2.79 ± 0.26	2.69 ± 0.26	4.37 ± 1.17
	2nd month	2.52 ± 0.30	2.41 ± 0.26	3.99 ± 1.19
	4th month	2.42 ± 0.30	2.31 ± 0.27	3.96 ± 1.20
B	Base line	3.19 ± 0.27	2.77 ± 0.27	5.64 ± 0.60
	2nd month	3.05 ± 0.27	2.63 ± 0.27	5.46 ± 0.62
	4th month	3.03 ± 0.27	2.62 ± 0.27	5.40 ± 0.63
C	Base line	2.76 ± 0.30	2.64 ± 0.24	4.08 ± 1.19
	2nd month	2.46 ± 0.31	2.35 ± 0.25	3.20 ± 1.19
	4th month	2.36 ± 0.30	2.24 ± 0.26	2.88 ± 1.18
D	Base line	2.84 ± 0.43	2.65 ± 0.26	5.64 ± 0.72
	2nd month	2.74 ± 0.43	2.58 ± 0.27	4.84 ± 0.72
	4th month	2.72 ± 0.43	2.56 ± 0.28	4.52 ± 0.73

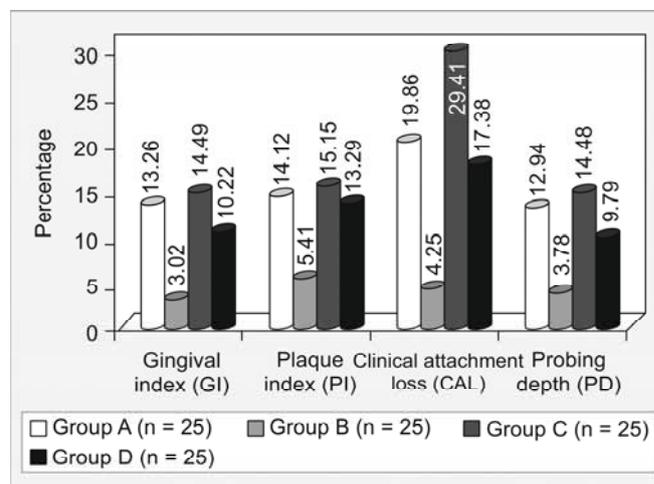
GI: Gingival index; PI: Plaque index; CAL: Clinical attachment level

that the mean values of all clinical parameters were significantly decreased more in group C, followed by group A, D and B. Thus, it was seen that group C is more consistent/reliable than other three groups in relation to clinical parameters considered in the study.

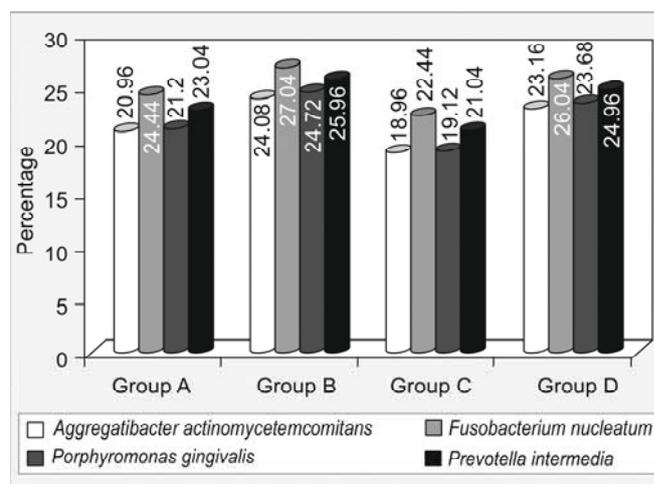
Microbiological Observations

Distribution of mean and standard deviation values of all the microbiological parameters of all the groups were illustrated in Table 3. The microbiological parameters (*Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Prevotella intermedia*) of experimental period, i.e. baseline, 2nd and 4th month were recorded and compared. Results showed a highly significant change with regard to improvement in the microbiological parameters from the baseline when compared to the 2nd and 4th month. By applying Student's unpaired 't' test there was a highly significant difference between mean values of microbiological parameters, Aa, Fn, Pg and Pi when all groups compared each other, (i.e. $p < 0.001$) as illustrated in Graph 3. Thus, it was seen that Group C showed more decreased (fall) of microbiological parameters than other groups in the study. That is decrease in the microbiological parameters was more/higher in group C followed by groups A, D and B. By applying Tukey-Kramer multiple comparison test there was a significant decrease in the mean values of microbiological parameters Aa, Fn, Pg and Pi in all four groups compared together (i.e. $p < 0.0001$) where value of 'F' = 4.408, $p < 0.0001$, very highly significant. It was observed that group C showed highest percentage decrease of microbiological parameters from baseline to 4th month in the entire recordings as illustrated in Graph 4. The percentage decrease of Aa, Fn, Pg and Pi from baseline to 4th month in group C was 26.74, 26.56, 28.97 and 26.84%. This was followed by group A and the percentage decrease of Aa, Fn, Pg and

Pi from baseline to 4th month was 25.58, 19.43, 20.26 and 22.89%. There was 24.12, 17.94, 19.68 and 21.31% percentage decrease of Aa, Fn, Pg and Pi from baseline to 4th month in group D. The last with least number of percentage decreases in microbiological parameters from



Graph 2: Percentage decrease of all clinical parameters from baseline to 4 months duration in all the groups under this study

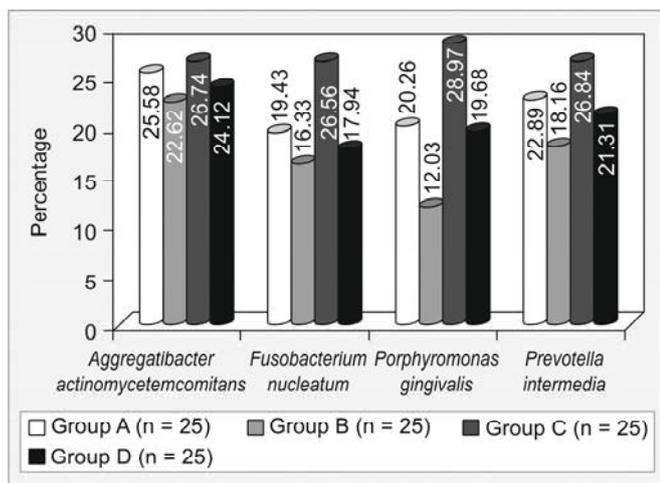


Graph 3: Comparison of mean values of all the microbiological parameters at 4 months duration in all the groups under this study

Table 3: Distribution of mean and standard deviation values (mean \pm SD) of all the microbiological parameters at baseline, 2nd and 4th month

Groups	Observation	Aa	Fn	Pg	Pi
A	Baseline	25.88 \pm 4.62	32.32 \pm 5.81	30.40 \pm 6.09	28.76 \pm 4.64
	2nd month	23.36 \pm 4.37	27.52 \pm 5.59	25.04 \pm 5.26	26.04 \pm 4.38
	4th month	20.96 \pm 4.31	24.44 \pm 6.32	21.20 \pm 3.37	23.04 \pm 4.35
B	Baseline	31.12 \pm 7.49	32.32 \pm 5.81	30.40 \pm 6.09	31.72 \pm 6.19
	2nd month	26.84 \pm 7.23	29.52 \pm 5.59	27.04 \pm 5.25	28.52 \pm 5.94
	4th month	24.08 \pm 7.32	27.04 \pm 5.83	24.72 \pm 4.95	25.96 \pm 5.79
C	Baseline	25.88 \pm 4.62	28.40 \pm 6.49	26.92 \pm 5.14	28.76 \pm 4.64
	2nd month	20.48 \pm 6.65	23.32 \pm 6.42	21.32 \pm 4.74	23.04 \pm 4.38
	4th month	18.96 \pm 4.31	22.44 \pm 3.62	19.12 \pm 3.45	21.04 \pm 4.35
D	Baseline	31.12 \pm 7.49	28.40 \pm 6.49	26.92 \pm 5.14	31.72 \pm 6.19
	2nd month	24.92 \pm 7.07	26.32 \pm 6.42	24.28 \pm 4.73	26.52 \pm 5.94
	4th month	23.16 \pm 7.15	26.04 \pm 5.83	23.68 \pm 4.98	24.96 \pm 5.79

Aa: *Aggregatibacter actinomycetemcomitans*; Fn: *Fusobacterium nucleatum*; Pg: *Porphyromonas gingivalis*; Pi: *Prevotella intermedia*



Graph 4: Percentage decrease of all microbiological parameters from baseline to 4 months duration in all the groups under this study

baseline to 4th month was observed in group B with 22.62, 16.33, 12.03 and 18.16% percentage values of Aa, Fn, Pg and Pi. It is concluded that the mean values of all microbiological parameters are significantly decreased more in group C, followed by group A, D and B. Thus, it was seen that group C was more consistent/reliable than other three groups in relation to microbiological parameters considered in the study.

DISCUSSION

The significant clinical and microbiological improvement in group C subjects (chlorine dioxide based toothpaste and mouthrinse along with dietary supplement of CoQ10) support that the hypothesis that sodium chlorite (Stabilized chlorine dioxide) may acts as a strong ingredient to restrict the proliferation of subgingival anaerobic microbiota via oxygenation and neutralization of toxins (Bacterial proteolytic enzymes) produces by the bacteria in the oral cavity. The stabilized chlorine dioxide based products (Oxyfresh Power Paste and Oxyfresh Power Rinse) also destroy the volatile sulfide compounds, which further reduce the triggering of gingival inflammation. The key benefits for these products also include nonstaining, alcohol free, nonirritating, no taste alterations, and sodium lauryl sulfate free (Foaming agent in toothpaste that initiate canker sore).

This study also revealed that the bactericidal activity of stabilized chlorine dioxide oral rinse (Oxyfresh Power Rinse) has marked bactericidal effects against with pathogens of periodontitis, i.e. Aa, Fn, Pg and Pi. These results are consistent with previous studies evaluating a stabilized chlorine dioxide oral rinse against polymicrobial suspensions and biofilm environments.^{9,10} The zinc acetate with xylitol further prevents the colonization of initial plaque formation and removes halitosis causing volatile organic compounds.

A deficiency of CoQ10 at its enzyme sites in gingival tissue may exist independently of and/or because of

periodontal disease.¹¹ If a deficiency of CoQ10 existed in gingival tissue for nutritional causes and independently of periodontal disease, then the advent of periodontal disease could enhance the gingival deficiency of CoQ10. In such patients, oral dental treatment and oral hygiene could correct the plaque and calculus, but not that part of the deficiency of CoQ10 due to systemic cause; therapy with CoQ10 can be included with the oral hygiene for an improved treatment of this type of periodontal disease.¹²

The specific activity of succinic dehydrogenase-coenzyme Q10 reductase in gingival tissues from patients with periodontal disease against normal periodontal tissues has been evaluated using biopsies, which showed a deficiency of CoQ10 in patients with periodontal disease. On exogenous CoQ10 administration, an increase in the specific activity of this mitochondrial enzyme was found in deficient patients.¹²⁻¹⁵

This study proved that when we supplement CoQ10 (Oxyfresh CoQ10 Complex) within chlorine dioxide based products (toothpaste and mouthrinse) there was synergistic effect on oral health gains as seen in group C compared with group A. Also when CoQ10 (Oxyfresh CoQ10 Complex) was supplemented with nonchlorine dioxide based product (toothpaste and mouthrinse), noticeable readings were noted. Many clinical trials with oral administration of CoQ10 to patients with periodontal disease have been conducted. Similar to our study, previous studies have also shown that oral administration of CoQ10 increases the concentration of CoQ10 in the diseased gingiva and effectively suppresses advanced periodontal inflammation^{14,16,17} and periodontal microorganisms. The CoQ10 nutritional supplement synergizes the effect and acting as potent antioxidant to counter the free reactive oxygen species released during the active phase of periodontal inflammation. This study concluded the fact that when we supplement CoQ10 with oral hygiene practice in both chlorine dioxide and nonchlorine dioxide groups, remarkable benefits in term of both clinical and microbiological parameters were noted. Our results were supported by previous study where periodontal score was decreased concluding that CoQ10 should be considered as an adjunct for the treatment of periodontitis in current dental practice.¹⁸

CONCLUSION

The comparative assessment revealed that sodium chlorite (Stabled Chlorine Dioxide) based dentifrice (Oxyfresh® Power Paste) and mouthwash (Oxyfresh® Power Rinse) has an edge over the conventional based dentifrice and mouthwash due to the above mentioned hypothesis and mechanism of the system that focus on the oxygenation of anaerobic environment and lead to disruption of the biofilm.

This study also suggested that sodium chlorite (Stabled Chlorine Dioxide) will significantly lowers bacterial count of all key periodontal pathogens considered in the study criteria, i.e. *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Prevotella intermedia*. The result also supports the beneficial effects of CoQ10 nutritional supplements in improving periodontal health. The results of this study were based upon normal home use of the products according to the manufacturers' instructions and coupled with specific instructions on proper tooth brushing and rinsing. Additional controlled studies should separate the paste from the rinse to determine if the sole use of either can generate clinical and microbiological improvements. In conclusion, both the stabilized chlorine dioxide toothpaste and rinse regimen and the phenol-related rinse regimen resulted in measurable clinical improvements with respect to gingival index, plaque scores and attachment level during the 4 months observation period. These findings suggest that there were significant periodontal benefits in using a stabilized chlorine dioxide toothpaste and rinse regimen along with nutritional supplement of CoQ10. Well-designed clinical trials will need to be conducted to adequately assess the effect of chlorine dioxide based products (tooth paste and mouthwash) on oral flora over a longer period of time. Then we will be able to recognize truly clinically noteworthy changes in the composition of flora that could cause oral disease. Further, more longitudinal studies with more number of patients for longer duration should be done to evaluate the long-term effect of chlorine dioxide based products (toothpaste and mouthwash) with potential benefits of CoQ10 supplements.

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