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RESEARCH ARTICLE

"ANTIBACTERIAL ACTIVITY OF 10% CARBAMIDE PEROXIDE BLEACHING AGENTS ON PERIODONTAL PATHOGENS" – AN IN VITRO STUDY

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ABSTRACT

Aim: The aim of this study was to compare and evaluate the antibacterial activity of commercially available 10% carbamide peroxide bleaching agents on periodontal pathogenic bacteria with 0.2% chlorhexidine gluconate.

Material & Methods: Two commercially available bleaching materials containing 10% Carbamide peroxide were selected for the study against Chlorhexidine solution (0.2%). Different bacterial stock were used. Wells each with a diameter of 5 mm were punched into each of the agar plates and filled with either one of the commercial bleaching materials or with Chlorhexidine solution (0.2%) to test for susceptibility test. All plates were incubated for 24 to 48 h at 37°C in a candle jar. The diameter of each zone of inhibition was measured, and the mean was calculated.

Results: Susceptibility tests were performed on disk sensitivity (DST) agar used routinely for antibiogram tests showed that Chlorhexidine group showed the highest mean value of Prevotella intermedia (23.67) and least in Actinobacillus actinomycetem comitans (20.33) with p value of 0.869. Opalescence Group using one way ANOVA test showed the mean value of Fusobacterium nucleatum (18.33) and least in Porphyromonas gingivalis with test value of 3.777 and p value of 0.027. Dr.collins group showed the highest mean value of Fusobacterium nucleatum (12.67) and no against Prevotellaintermedia (0) with a test value of 169.7 and p value of <0.001.

Conclusion: Chlorhexidine (0.2%) displayed higher antibacterial effects on the periodontal pathogens with the highest mean value against porphyromonas gingivalis at 23.67 than both the tested bleaching materials containing 10% carbamide peroxide in this in-vitro study showing that chlorhexidine would thus be more beneficial for the prevention of periodontal diseases.

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INTRODUCTION

Periodontitis is defined as "an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both". The gram negative pathogenic organisms which are actively responsible for periodontal etiology are porphyromonas gingivalis, prevotella intermedia, actinobacillus actinomycetemcomitans, fusobacterium nucleatum and increase in these gram negative anaerobe or facultative bacteria present in the subgingival biofilm leads to further periodontal destruction and also these pathogens have the ability to invade gingival tissues and interact with the host immnune response (wolf *et al*., 1994). Certain bleaching agents such as hydrogen peroxide (HP) or carbamide peroxide (CP), used for esthetic purposes, have gained worldwide popularity because of their efficacy in whitening teeth at a very low cost and minimal invasion of dental hard and soft tissues (Haywood *et al*., 1991). CP bleaching systems consist of hydrogen peroxide coupled to urea in an anhydrous glycerin base (Yarborough DK *et al*., 1990). Hydrogen peroxide, the active ingredient in CP, is a nontoxic, nonallergic antimicrobial agent capable of killing a broad range of microorganisms (Tartakow D J *et al*., 1978). Urea is a nontoxic bacteriostatic substance capable of dissolving necrotic tissue, allowing wounds to heal more quickly (Shipman *et al*., 1981).urea peroxide in anhydrous glycerol has showed its effectiveness in reducing plaque and gingivitis scores and also caries formation (Zinner *et al*., 1978). Vital bleaching of teeth with hydrogen peroxide under controlled clinical conditions has widely been used for teeth whitening. Nevertheless several reports of gingival irritation

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and ulceration in some patients suggest that bleaching agents, under certain circumstances, promote toxic effects on oral cells and in human gingival fibroblasts (Rotstein*et al*., 1991 and Munro *et al*., 2006)

Table 1. Bleaching materials used in the study

Material	Active ingredient
Opalescence	10% carbamide peroxide
Dr. Collins	10% carbamide peroxide

Table 2. One way Anova with posthoctukey test: 4 groups

		N	Mean	Std. Deviation	Statistics/ mean squares	df2(welch) / F(Anova)	p value
Group I – Chlorhexidine	*Porphyromonasgingivalis*	3	22.67	2.517	6.083	2.355	0.148
	Prevotellaintermedia	3	23.67	1.155			
	Actinobacillusactinomycetemcomitans	3	20.33	0.577			
	Fusobacteriumnucleatum	3	21.67	1.528			
	Total	12	22.08	1.881			
Group II - Opalescence Group	*Porphyromonasgingivalis*	3	11	1.732	10.326	3.777	0.027
	Prevotellaintermedia	3	16	6.083			
	Actinobacillusactinomycetemcomitans	3	12.67	0.577			
	Fusobacteriumnucleatum	3	18.33	1.528			
	Total	12	14.5	4.079			
group III- Dr. collins	*Porphyromonasgingivalis*	3	11	1.732	141.417	169.7	≤0.00
	Prevotellaintermedia	3	0	0			
	Actinobacillusactinomycetemcomitans	3	0	0			
	Fusobacteriumnucleatum	3	12.67	0.577			
	Total	12	5.92	6.259			

It has also been seen that hydrogen ions are produced in the process of hydrogen peroxide break down, which may produce a relatively acidicenvironment with the bleaching procedures and might affect the surface and subsurface integrity of hard and soft tissues (Xu *et al*., 2011). The effect of 10% CP solution (Proxigel) on the salivary levels of S. mutans and lacto- bacilli was studied and it was reported that salivary levels of lactobacilli were reduced, but this solution had no effect on Mutans streptococci (Bentley and Leonard *et al*). Chlorhexidine (CHX), a cationic bis-biguanide and has broad-spectrum antibacterial activityand is considered a gold standard among antibacterial agents used in periodontal therapy (Davies *et al*., 1954). The primary mechanism of action of CHX is membrane disruption, causing concentration-dependent growth inhibition and cell death (Hugo *et al*., 1966). Secondary interactions causing inhibition of proteolytic and glycosidic enzymes may also be significant (Hastings *et al*., 2000). The cationic nature of CHX enables it to bind to tooth surfaces and oral mucosa, reducing pellicle formation and increasing substantivity through controlled release of the agent (Bonesvoll *et al*., 1974). However, CHX has been reported to have a number of side effects like brown discoloration of teeth, salt taste perturbation, oral mucosal erosions, and enhanced supragingival calculus formation, which limit its long term use (Eley *et al*., 1999). Very fewstudies have been conducted on the antibacterial efficacy of 10% carbamide peroxide on periodontal pathogens and so, the purpose of the present in vitro study was to examine the antibacterial effect of two commercial 10% CP bleaching agents against specific periodontal pathogens.

METHODS AND MATERIALS

Two commercially available bleaching materials containing 10% carbamide peroxide were selected for the study (Table 1). A 0.2% Chlorhexidine solution was included as a positive control. The following bacteria from a stock collection were used in the experiments: porphyromonas gingivalis, prevotella intermedia, aggregatibacter actinoimycetemcomitans, fusobacterium nucleatum. Strains were reconstituted from lyophilization and grown anaerobically in Nutrient Broth (Hi Media).

Turbidity of the bacterial suspensions was adjusted to a #0.5 McFarland nephelometer standard and used as an inoculum. Susceptibility tests were performed on disk sensitivity (DST) agar used routinely for antibiogram tests. DST medium (12 ml) was poured into 7-cm Petri dishes with an agar depth of 4.5 to 5 mm. Three drops of inoculum were seeded onto the surfaces of plates by a cotton swab. Surfaces were then allowed to dry at 37°C for 30 min. Wells each with a diameter of 5 mm were punched into each of the agar plates and filled with either one of the commercial bleaching materials or with 0.2% chlorhexidine solution. All plates were incubated for 24 to 48 h at 37°C in a candle jar. The diameter of each zone of inhibition was measured, and the mean was calculated. Tests were repeated three times, followed by similar calculations. The diameter of the zone of inhibition will determine the effectivenesss of the antibiotic; the larger the diameter, the greater wll be the sensitivity of the bacterium to the antibiotic. The clear region around the paper disc saturated with an antimicrobial agent on the agar surface is the zone of inhibition. The clear region is an indication of the absence, or the effective inhibition, of microbial growth by the antimicrobial agent.

Statistical Analysis

Statistical analysis was done for intergroup and intragroup comparison using one way anova test and posthoc tukey test

RESULTS

All the 3 materialsdemostrated good antibacterial effect but chlorhexidine showed highest antibacterial effect against propyromonasgingivalis. chlorhexidine showed largest inhibition zone against prevotellaintermedia & porphyromonas gingivalis; while opalescence showed largest inhibition zone for fusobacteriumnucleatum & prevotellaintermedia and Dr. Collins showed largest inhibition zone for fusobacterium nucleatum & porphyromonasgingivalis. The mean values of

the diameter of inhibition zones for each bleaching material and the 0.2% chlorhexidine solution shown in Table 2 and Table 3.

concentrations, it damages bacterial membranes without inducing mutation. HP also triggers the release of extracellular DNA without autolysis, which promotes intra-species cell-to-

Table 3. Posthoctukey test for subgroup analysis

Dependent Variable	Comparison group	Compared with	Mean difference	Std. Error	P value
Group I - Chlorhexidine	*Porphyromonasgingivalis*	prevotellaintermedia	-1	1.312	0.869
		actinobacillusactinomycetemcomitans	2.333	1.312	0.349
		fusobacteriumnucleatum	1	1.312	0.869
	Prevotellaintermedia	actinobacillusactinomycetemcomitans	3.333	1.312	0.127
		fusobacteriumnucleatum	2	1.312	0.468
	Actinobacillusactinomycetemcomitans	fusobacteriumnucleatum	-1.333	1.312	0.745
Group II - Opalescence Group	*Porphyromonasgingivalis*	prevotellaintermedia	-5	2.667	0.31
		actinobacillusactinomycetemcomitans	-1.667	2.667	0.921
		fusobacteriumnucleatum	-7.333	2.667	0.095
	Prevotellaintermedia	actinobacillusactinomycetemcomitans	3.333	2.667	0.616
		fusobacteriumnucleatum	-2.333	2.667	0.818
	Actinobacillusactinomycetemcomitans	fusobacteriumnucleatum	-5.667	2.667	0.224
group III- Dr. Collins	*Porphyromonasgingivalis*	prevotellaintermedia	11.000*	0.745	<0.001
		actinobacillusactinomycetemcomitans	11.000*	0.745	<0.001
		fusobacteriumnucleatum	-1.667	0.745	0.193
	Prevotellaintermedia	actinobacillusactinomycetemcomitans	0	0.745	1
		fusobacteriumnucleatum	-12.667*	0.745	<0.001
	Actinobacillusactinomycetemcomitans	fusobacteriumnucleatum	-12.667*	0.745	<0.001

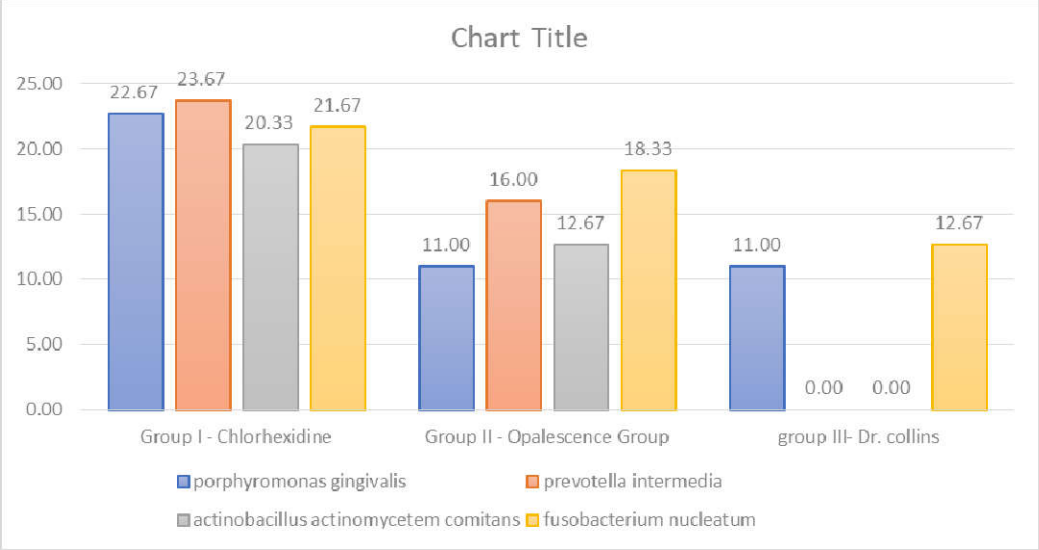

Figure 1. Antibiotic susceptibility test of 10% carbamide peroxide bleaching agent against 0.2% chlorhexidinegluconate

DISCUSSION

Antimicrobial activity has been extensively studied in recent decades due to to the persistence of antimicrobial resistence in bacterial population. Thus, there has been made many efforts to search for new antibacterial agents used in dentistry, mainly in response to the staggering concern of consumers over the safety of synthetic products. Bleaching agents are also widely used among dentists in the clinical practice. Carbamide (urea) peroxide (CP) decomposes into one part hydrogen peroxide (HP) and two parts urea. Urea further decomposes into ammonia and carbon dioxide via urease activity. HP and CP are acidic, at pH's 4.5 and 5.3, respectively, and exert inhibitory effects on acidophilic pathogens, such as S. mutans, through the release of ammonia and elevation of the pH when CP decomposes. Hydrogen peroxide (HP) was first reported for intraoral use in 1913 to decrease plaque formation, and it has been the treatment of choice for acute necrotising ulcerative gingivitis and pericoronitis. At low concentrations, HP damages DNA and proteins, while at higher (30 mM) cell adherence and aggregation within the structure of the biofilm. Effervescence from the 10% CP rinse dislodged the biofilm almost completely after 1 min, offering a clear advantage to the more passive CHX, which showed a comparable bactericidal effect. (Chao Shu Yao *et al*., 2013). In an in-vivo study (Almas *et al*., 2003), following application of 10% CP in custom trays, demonstrated a reduction in bleeding on probing, Plaque Index and Gingival Index scores. However, no appropriate controls were used. A double blind, randomized, controlled, parallel group clinical trial (Brunton *et al*., 2004) demonstrated that self-application of CP (at a concentration of 16−18%) resulted in a statistically significant reduction in gingival scores ($P < 0.001$). A study by Zinner *et al* proposed that alterations in plaque microflora, debridement properties of peroxides and the ability of the CP to increase availability of oxygen thus promoting tissue healing, resulting in reduction of gingivitis (Zinner *et al*., 1978). One of the most common antimicrobials prescribed for patients is chlorhexidine digluconate (CHX) as it has excellent

substantivity resulting in activity for several hours. Carbamide peroxide may have properties similar or even superior to that of CHX. In contrary to the present study, an in vitro study (Yao *et al*., 2013) showed that 10% CP bleaching agent demonstrated a superior bactericidal and dislodging effect on oral biofilm cultured within an in vitro anaerobic model compared to a control and 1% chlorhexidine solution. Bleaching agents are cytotoxic to human gingival fibroblasts, increasing the effects on cell viability and morphology, and on the proliferation and production of fibronectin and collagen (Tipton *et al*., 1995). In an in vitro study by Koulaouzidou *et al*, investigated the cytotoxic effect of a bleaching agent on 2 fibroblast cell lines and found that both were sensitive to urea peroxide and suggested that the potential damage to oral tissues in vivo may be considerable because of the direct andlong-term exposure of the tissues to the bleaching agents (Koulaouzidou *et al*., 1998). After a thorough study of literature and to the best of our knowledge, there are no in-vitro studies on 10% carbamide peroxide, to confirm its antibacterial activity on periodontal pathogens. In the present study ,when Comparing Group I, Chlorhexidine using one way ANOVA test was done the mean value of prevotella intermedia (23.67) is highest followed by porphyromonas gingivalis (22.67), fusobacterium nucleatum (21.67) least in actinobacillus actinomycetemcomitans (20.33) but the difference wasnot statistically Significant. In Group II - Opalescence Group using one way ANOVA test showed that the mean value of fusobacterium nucleatum (18.33) was highest followed by prevotella intermedia (16), actinobacillus actinomycetemcomitans (12.67) least in porphyromonas gingivalis (11) and here the difference was statistically Significant. And in group III- Dr.collins using one way ANOVA test showed that the mean value of fusobacterium nucleatum (12.67) was highest followed by porphyromonas gingivalis (11), prevotella intermedia (0) least in prevotella intermedia (0) and the difference is statistically Significant.

Conclusion

In the present study, 0.2% chlorhexidine solution was included as a control solution against which the other test substances were compared. Chlorhexidine displayed higher antibacterial effects than both the tested bleaching materials. This, in-vitro study demonstrates that commercially marketed 10% CP bleaching materials do not vary greatly in their ability to act on the growth of periodontal pathogenic organisms. These experimental findings, however, only provide data that are useful for assessment of in vitro antibacterial effects. In vivo models that will account for many variables to which these solutions are exposed under clinical conditions should be sought.

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