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COMPARATIVE EVALUATION OF INHIBITORY EFFECT OF BLUE-M ORAL GEL VERSUS HEXIGEL ON *PORPHYROMONAS GINGIVALIS*: AN IN-VITRO STUDY

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ABSTRACT

Introduction: Periodontitis is a multifactorial chronic inflammatory disease associated with altered dental biofilm that causes progressive destruction of supporting tissues of teeth where microorganisms play crucial role. Among these *Porphyromonas gingivalis* is major Gram-negative anaerobic bacterium involved in pathogenesis of periodontitis, which has numerous virulence factors. Traditionally, this disease is treated with basic periodontal procedures along with the use of chlorhexidine digluconate. Oxygen is essential nutrient for multiple processes including oxidative killing of bacteria, re-epithelialization, angiogenesis, and collagen synthesis. Recently, new product that releases oxygen has been introduced. Considering these characteristics, Blue – M Oral gel is compared with Hexigel to evaluate the beneficial effects on periopathogens. **Objective:** To compare inhibitory effects of Blue – M Oral gel and Hexigel against *Porphyromonas gingivalis*. **Methodology:** In this in- vitro study, the inhibitory effects of Blue – M Oral gel will be studied on *P. gingivalis* culture and compared to Hexigel and distilled water by using agar disc diffusion method. After incubation, diameter of halos of inhibition of bacterial growth around the paper discs will be measured and compared. **Results:** The intergroup comparison of antimicrobial effects of Blue M Oral Gel and Hexigel against *P. gingivalis* showed a statistically highly significant (p value < 0.001). **Conclusion:** Hexigel acts effectively against *P. gingivalis*, hence remains to be the gold standard while Blue -M oral gel is not effective antimicrobial agent within the limitations of study.

KEYWORDS: Blue -M oral gel, Hexigel, *P. gingivalis,* Antimicrobial effect.

INTRODUCTION

Periodontitis is a multifactorial chronic inflammatory disease associated with an altered dental biofilm that causes progressive destruction of the supporting tissues of the teeth.[1] It is now generally agreed that the formation of plaque on teeth represents a massive accumulation of bacteria already present in the oral cavity and that bacterial colonization plays an essential role in the initiation and progression of periodontal disease.[2] Among these, *Porphyromonas gingivalis* a Gram-negative oral anaerobic bacterium is involved in the pathogenesis of periodontitis and has numerous virulence factors, capable of inducing intense tissue destruction in periodontal infections.[3]

Successful periodontal therapy is dependent on anti-infective procedures aimed at eliminating pathogenic organisms found in dental plaque associated with the tooth surface and within other niches in the oral cavity.[4] Anti-infective therapy includes both mechanical and chemotherapeutic approaches to minimize or eliminate microbial biofilm (bacterial plaque), the primary etiology of gingivitis and periodontitis.[5] Mechanical plaque control is the mainstay for prevention of oral diseases, but it requires patient cooperation and motivation; therefore, chemical plaque control agents act as useful adjuvants for achieving the desired results.[6] Chlorhexidine is considered the gold standard. However, perpetual use of Chlorhexidine should be avoided as it possess certain drawbacks such as staining of teeth and restorations, desquamation of oral mucosa, altered taste sensation and enhanced supragingival calculus formation.[7] So, there is need for exploration of newer products with similar antimicrobial efficacy and lesser adverse effects.

Oxygen is an essential nutrient for cellular metabolism, especially energy production. It is involved in multiple processes including oxidative killing of bacteria, re-epithelialization, angiogenesis, and collagen synthesis.[8] A team of dental surgeons led by Dr. Peter Blijdorp in the Netherlands, developed a product based on active oxygen (Blue®M), with the intention of putting all the desirable properties of mouthwashes in just one product. Blue-m has in its composition sodium perborate, the glucose oxidase enzyme derived from honey, xylitol and lactoferrin. Applications in dentistry vary from use in inflammation of gums and ulcers, peri-implantitis, after various oral surgical procedures and a substitute for hypochlorite sodium during irrigation of root canals.[9]

Wound healing is an extremely complex process, which requires a variety of cells to increase its metabolic activity, resulting in oxygen demand.[10] Topical Oral Oxygen Therapy (OOT) is aimed at accelerating the healing process by ensuring neovascularization, removing toxins, stimulating the formation of new blood cells, increasing the production of stem cells, and eradicating bacteria.[11] OOT with Blue®M can be a substitute for CHX in postsurgical care, not showing the disadvantages observed with the use of CHX, especially in relation to the cytotoxic effect on gingival cells.[12]

As there is lack of evidences on oral oxygen therapy, the present study was conducted to evaluate the antibacterial property of Blue M oral gel against *Porhyromonas gingivalis.*

MATERIAL AND METHODS

The present study is an in- vitro microbiological study conducted in Maratha Mandal's Central Research Laboratory, Belgaum. In this study, the antibacterial activity against *Porphyromonas gingivalis* was examined by Well diffusion test on blood agar.

The inoculum was prepared using a loop and colonies were transferred to the test tube containing thioglycolate broth. The turbidity of the broth was then adjusted visually to equal that of a 0.5 McFarland turbidity standard that has been vortexed.

Within 15 minutes of adjusting the inoculum to a McFarland 0.5 turbidity standard, the entire surface of agar plate was swabbed six times, rotating plates approximately 60º between streaking to ensure even distribution. The inoculated plates were allowed to stand for at least 3 minutes but no longer than 15 minutes before making the wells.

For addition of the compound into plate, a hollow tube of 5mm diameter was taken and heated. It was then pressed above inoculated Agar plate and removed immediately by making a well in the plate. Likewise, three wells on each plate were made. With the help of micropipette 2 mg of each compound (Group 1 Blue-M oral gel and Group 2 Hexigel) were added in each plate along with 50µL of distilled water which was taken as negative control in the third well. The test was repeated for 6 times.

Within 15 minutes of compound application, the inoculated plates were incubated for 48-72 hours at 37 ºc under anaerobic condition in the anaerobic jar. If the lawn of growth was confluent or nearly confluent, only then the reading of the plates were carried out. The diameter of inhibition zone was measured to the nearest whole millimeter by holding the measuring device and intergroup comparison was done.

STATISTICAL ANALYSIS

To assess the reliability of the data obtained, statistical analysis was performed using Statistical Package for Social science (SPSS) version 21 for Windows. Descriptive quantitative data was expressed in mean and standard deviation respectively. Data normality was checked by using Shapiro – Wilk test. Confidence interval was set at 95% and probability of alpha error (level of significance) set at 5%. Power of the study was set at 80%. Overall intergroup comparison among three groups in relation to diameter of halo of inhibition against *P. gingivalis* was done using Chi square test.

RESULTS

The bacterial growth of *P. gingivalis* was absent in presence of Hexigel as the mean diameter of inhibition halo was 24.5mm, while in case of Blue-M oral gel, abundant colonies of *P. gingivalis* were observed suggestive of resistance of *P. gingivalis* which is equivalent to antibacterial effect of (Graph1). The diameter of inhibitory halos obtained is shown in Table 1, Figure 1. The intergroup comparison of effects of Blue M Oral Gel (Group 1), Chlorhexidine digluconate gel (Group 2), Distilled water (Group 3) on *P. gingivalis* shows a p value < 0.001 which is statistically highly significant. (Table2, Graph 2).

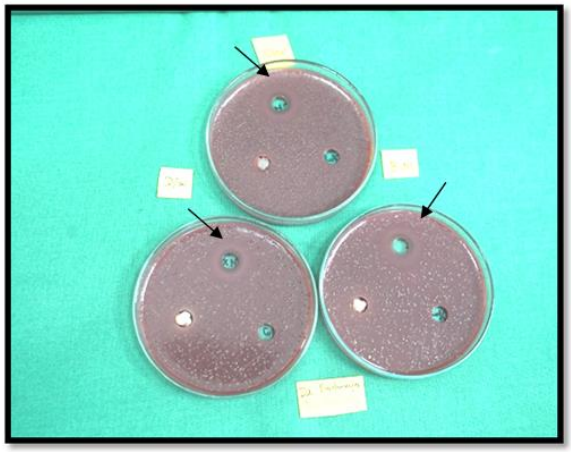
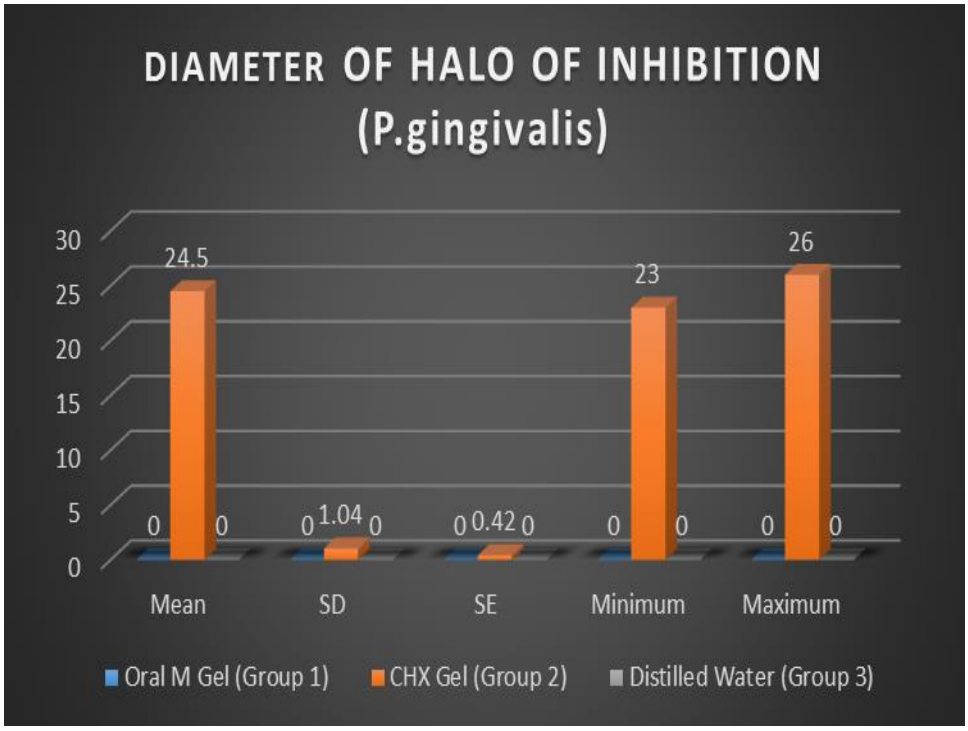


Figure 1: Inhibition hollow around Chlorhexidine group (shown by black arrow).



Graph 1: Diameter of halo of *P. gingivalis*.

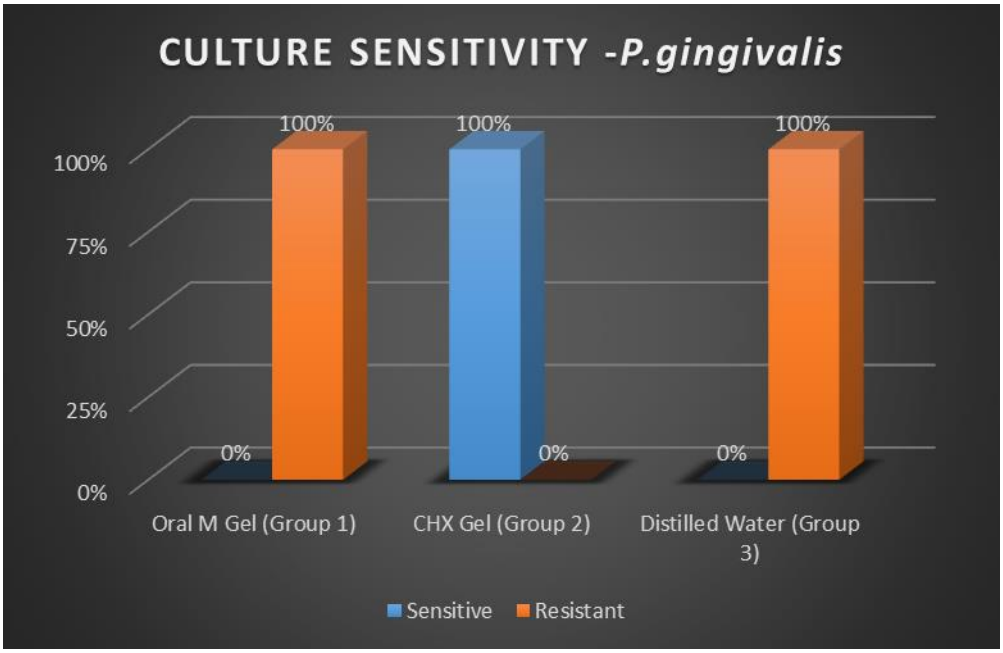
Table 1: Descriptive statistics of diameter of halo of inhibition of bacterial growth (sensistive) against *P. gingivalis* recorded in mm.

In mm	Blue M Oral Gel (Group 1)	Chlorhexidine Gel (Group 2)	Distilled Water (Group 3)
Sample 1	Resistant	25	Resistant
Sample 2	Resistant	24	Resistant
Sample 3	Resistant	25	Resistant
Sample 4	Resistant	23	Resistant
Sample 5	Resistant	24	Resistant
Sample 6	Resistant	26	Resistant
Mean	----	24.5	----
SD	----	1.04	----
SE	----	0.42	----
Minimum	----	23.0	----
Maximum	----	26.0	----

Table 2: Comparison of effects of Blue M Oral Gel (Group 1), Chlorhexidine digluconate gel (Group 2), Distilled water (Group 3) on *P. gingivalis.*

P. gingivalis	**Sensitive n (%)**	**Resistant n (%)**
Oral M Gel (Group 1) (n=6)	0/6 (0%)	6/6 (100%)
Chlorhexidine Gel (Group 2) (n=6)	6/6 (100%)	0/6 (0%)
Distilled Water (Group 3) (n=6)	0/6 (0%)	6/6 (100%)
Overall Group (1 vs 2 vs 3)	Chi = 18.0, p<0.001**(HS)	
Oral M vs CHX	Chi = 12.0, p =0.001*(S)	
Oral M vs DW	Chi =0.0, p =1.000 (NS)	
CHX vs DW	Chi = 12.0, p =0.001* (S)	

p>0.05 – no significant difference *p<0.05 – significant **p<0.001 – highly significant



Graph 2: Intergroup comparison among different groups against *P. gingivalis.*

DISCUSSION

It is well quoted that **"What oxygen is to the lungs such is plaque control for better oral hygiene"**. This delineates that plaque control is imperative to limit the progression of periodontal disease. It has been known that periodontitis is a chronic inflammatory, multifactorial, polymicrobial disease commenced by built up of dental plaque biofilm and sustained by down regulated immune response and usually preceded by gingivitis resulting in irreversible destruction of supporting tissues surrounding the tooth.[5] It is well acknowledged that the initiation and progression of the disease is not only due to the presence of bacterial strains pathogenic for the periodontium but also due to absence / minimal proportion of beneficial commensals in the susceptible host.[13] As the disease progresses, the resident Gram -ve anerobic bacteria interact in the host inflammatory reaction leading to a lower oxygen or hypoxic environment within the affected sulcus or periodontal pocket.[14] Certain periodontopathogens like *P. gingivalis* under hypoxia increase the oxidative stress in periodontal ligament fibroblast and induces a collapse of protective mechanism causing an increase in ROS and progression of inflammatory oral diseases.[15] Reactive oxygen species including superoxide, Hydrogen peroxide, hydroxyl anions induces damage to DNA, proteins & lipids in host tissues. Thus, a decline in oxidative stress by using oxygen releasing products would be of potential therapeutic value in the improvement of periodontitis.[16]

Recently, attention has been drawn to the oxygen delivering gel, commercially available as Blue-M oral gel. It is used in various dental fields as it possess antimicrobial & anti- inflammatory properties which prevents formation of plaque biofilm as well as improves the rate of wound healing.[17] The basic mechanism of Blue-M is controlled delivery of active oxygen i.e. hydrogen peroxide to the site of treatment. This occurs when Sodium perborate comes in contact with water, it creates a chemical process of hydrolysis, the end products being hydrogen peroxide and boric acid. Hydrogen peroxide enhances collagen mRNA abundance and other cytokines and growth factors which depend on oxygen supply. Hydrogen peroxide further reacts with saline and releases nascent oxygen.[16]

The present study was an in-vitro, microbiological study which emphasized on assessing inhibitory effect of Blue-M oral gel on *Porphyromonas gingivalis* and comparing it with Chlorhexidine digluconate gel (Hexigel) by Agar diffusion method. In this study, blood agar was used as it serves as a nutritious, non selective medium allowing cultivation of not only fastidious anaerobes but also aerobic and microaerophilic microorganisms. This media braces typical pigment production by P. gingivalis and various other anaerobic micro-organism. After pertinent incubation period, it was ascertained that an inhibitory halo against *P. gingivalis* was found with 1% Chlorhexidine digluconate gel (Hexigel). On contrary, there was no such inhibitory halo against *P. gingivalis* in presence of slow-oxygen releasing gel (Blue-M oral gel). Till date, a limited evidence exists regarding antibacterial property of Blue-M oral gel.

Amidst these, a study done by **T.M. Deliberador et al[18] (2020)** showed contradictory results. They found that Blue-M at a dose of 100% and 75% showed a similar result as that of Chlorhexidine ($p>0.05$). However, Blue-M at a concentration of 50% showed lower inhibition halo when compared to Chlorhexidine. Whereas in the present study, 100% Blue -M oral gel did not showed any antibacterial activity ($p<0.001$). This may be

attributed to the change in the methodology, as agar diffusion method was used in the above study.

Sparse clinical trials exist regarding the use of Blue-M oral gel as an adjunctive treatment of periodontitis. One such randomized split mouth clinical trial conducted by **Niveda & Kaarthikeyan[19] (2020)** compared the effects of oxygen releasing oral gel and Chlorhexidine gel in the treatment of periodontitis. They found a significant difference in reduction of probing pocket depth, clinical attachment level and bleeding on probing with scaling and root planing along with adjunctive use of Blue -M oral gel when compared to Chlorhexidine gel.

Another clinico-microbiological study conducted by **Kaul et al (2019)[20]** also showed equally effective and comparable effects of Chlorhexidine gel and Blue-M oral gel as an adjunct to non-surgical periodontal therapy. Signifcant reduction in gingival index and pocket depth was seen. This might be due to the fact that more active oxygen was delivered at the treated sites which improved the gingival inflammation and provided for a progressive and faster healing.

The substantivity of the Blue- M oral gel is still not known. Hence, necessitates further clinical research and trials.

Also, evidences exist on other properties of Blue-M oral gel having anti-inflammatory action, preventing formation of plaque biofilm, promoting teeth whitening and improving rate of wound healing.[17]

However, the present study was confined to assess only the antibacterial property. Thus, there is a prerequisite of further research to assess the effect of oxygen delivering agents and their various properties in the treatment of periodontitis. Also, there is a need to standardize the protocol regarding the use of Blue- M oral gel in terms of frequency and duration.

LIMITATIONS

The present study has nominal limitations, one of them being that it is an in- vitro study, which does not replicate the actual oral environment, hence necessity of further clinical trials is must. Also, it was limited to assess only one microorganism related to the periodontal disease, however enormous range of microorganisms are accountable for the disease condition. Thus, there is a need for evaluation of effect of Blue M oral gel on other potential pathogens.

CONCLUSION

- The effect of Blue- M Oral gel and distilled water was equivalent and showed no antibacterial effect against *Porphyromonas gingivalis*.
- While the Hexigel (Chlorhexidine digluconate gel) showed highly significant antibacterial effect against *P. gingivalis*, hence remains to be the gold standard.

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