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Antibacterial Efficacy of Oxygen Enriched Mouthwash in Patients Undergoing Dental Implant Placement by Real Time Polymerase Chain Reaction

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ABSTRACT

Aim: To evaluate the antibacterial efficacy of oxygen enriched mouthwash compared to chlorhexidine mouthwash in patients undergoing dental implant placement.

Materials and Methods: A total of 30 systemically healthy patients visiting Saveetha Dental College and Hospitals for dental implant placement between July 2022 and October 2022 were divided into two groups; Group A comprised of 15 patients, who were given chlorhexidine mouthwash and Group B composed 15 patients, who were given oxygen enriched mouthwash, postoperatively for 1 month following dental implant placement surgery. Plaque samples were collected prior to implant placement and 1 month after implant placement for both the groups. The collected plaque samples were subjected to real time polymerase chain reaction. Total bacterial copy-count and Porphyromonas gingivalis load were detected for both the groups.

Results: There was reduction in total bacterial copy-count in both the groups from baseline. However, the difference was not statistically significant (p=0.827). However there was more reduction in Porphyromonas gingivalis load among the patients who received oxygen enriched mouthwash and was statistically significant (p=0.000).

Conclusion: Oxygen enriched mouthwash can be used as a safe alternative to chlorhexidine in reducing the microbial load during the post operative healing period following dental implant placement.

Keywords: *Antimicrobial, Chlorhexidine, Mouthwash, Oxygen therapy*

INTRODUCTION

Dental implants are now the most accepted option of tooth replacement by patients worldwide. This growing popularity of dental implants requires proper healing of the edentulous site for its success. Importance of healing after implant placement is necessary as any microgaps or leaching of the oral microflora during osseointegration process might lead to the colonization of the bacteria in the surrounding peri-implant tissues which will in turn affect the osseointegration of the implant and lead to early failure of the dental implants.1,2 Wound healing without any pathogenic bacterial plaque is essential for the initial success of the implant.3

Oral microflora has the potential to alter wound healing. Gram-negative anaerobes, found in subgingival plaque, colonize the oral cavity after Gram-positive bacteria.4 The microbial flora in the peri-implant sulcus is similar to periodontal sulcus. In plaque, microorganisms show a shift from gram positive (primary colonizers) to gram negative (secondary colonizers). The shift in this microbial flora which turns the symbiotic association of host and microbe to pathogenic is also known as polymicrobial dysbiosis.5 These gram negative colonizers have known virulence factors that are capable of tissue destruction, host defense elusion and delay tissue healing, leading to peri-implantitis and failure of the implant.6

It has been found that oxygen is an effective substrate for wound healing. In tissues, oxygen is necessary for electron transport, oxidative phosphorylation, and energy production. Numerous physiological and pathological conditions can alter the amount of oxygen required by tissues.7 Lack of oxygen in the cells, or cellular hypoxia, can result in significant changes such as cell development, proliferation, and survival may be impacted by these changes, which can be immediate or gradual; impacting angiogenesis, metabolism, and pH regulation.8 The bacterial oxidative killing process, reepithelialization, angiogenesis, and collagen synthesis are few of the wound healing processes that depend on oxygen. Therefore, both topical and hyperbaric oxygen therapy have been studied as therapeutic modalities to promote wound healing.9 Infection prevention, increased reepithelialization, collagen synthesis via fibroblast growth induction, and angiogenesis are the potential effects of oxygen on wound healing. Topical oxygen therapy is much more practical because it can be done at home, less expensive, and has minimal side effects despite the fact that it is actually less toxic.10 Given the above, oxygen can play a role in reducing the severity of chronic inflammatory conditions such as peri-implantitis. Therefore in recent days, researchers have started introducing oxygen in forms such as gels, mouthwashes, in forms such as ozonated water gas and oils for its anti- bacterial properties.11

Oxygen enriched mouth composed of active ingredient sodium perborate, which is a mild, inexpensive and air-stable oxidizing agent which has high affinity for water. This breakdown of sodium perborate, an excellent disinfectant, leads to the formation of hydrogen peroxide which in turn breaks down into water and oxygen.12 Due to its substantivity property and broad antimicrobial spectrum, chlorhexidine has long been the gold standard among mouthwashes used in dentistry. Due to its inherent drawbacks, such as staining and taste sensation changes, there has been a search for a variety of alternatives to chlorhexidine.

Our team has extensive knowledge and research experience that has translated into high quality publications.13–22 Literature search reveals studies comparing oxygen derived products with chlorhexidine on halitosis, periodontitis, gingivitis etc.23–26 However, there are no studies to compare the efficacy of oxygen enriched mouthwash on peri-implant microflora. In this context, the purpose of this study was to clinically evaluate the antibacterial efficacy of an oxygen enriched mouthwash compared to chlorhexidine mouthwash by real time polymerase chain reaction (RT-PCR) in patients undergoing dental implant placement.

MATERIALS AND METHODS

Study population

The participants of the study were selected from the Department of Oral Implantology of Saveetha Dental College and Hospitals, Chennai, India.

The study was for a duration of 3 months. A total of 30 patients undergoing dental implant placement were selected for the study during the period of July 2022 to October 2022. The systemic health and periodontal status of each patient was recorded. Informed consent was taken prior to placement of the dental implants and the study was conducted with ethical clearance from the Institutional Scientific Review Board of Saveetha Dental College and Hospitals, Chennai.

Inclusion and exclusion criteria

The inclusion criteria for the following study was systemically healthy individuals, patients with one or two missing teeth irrespective of maxilla or mandible, periodontally healthy individuals without any prior history of periodontal treatment. Patients with any systemic illnesses such as diabetes, cardiovascular disorders or autoimmune disorders, patients receiving antibiotics, anticoagulants or anti-inflammatory medications, smokers, and lactating mothers were excluded. All patients received a complete scaling on their first visit, as well as oral hygiene instructions were given.

Implant placement protocol

After enrolling the patients based on the inclusion criteria, a coin toss was performed by one operator (A.A) and the patients were divided into two groups:

Group A- Patients receiving chlorhexidine mouthwash (Rexidin®) and

Group B- Patients receiving oxygen enriched mouthwash (BlueM®)

Implants of the same brand (Straumann Roxolid SLActive surface) were placed either in the maxillary or mandibular jaw in this study. Under strict aseptic protocols, implants were placed after sequential drilling by the same operator (I.R.). After implant and healing abutments placement, antibiotics and analgesics were prescribed to all patients undergoing this trial. Amoxicillin 500mg thrice daily for 5 days and Aceclofenac 100mg was prescribed.

Sample collection

Prior to implant placement, teeth adjacent to the implant site were isolated and subgingival plaque samples were collected using a Mini Five titanium curette (Hu-Freidy, USA) from all the patients. Similar protocol was followed for sample collection one month following implant placement from the healing abutments. The samples were stored in a tight eppendorf tube in Phosphate Buffered Saline (PBS) solution at -20 degree celsius till it was processed.

Microbiological analysis by RT-PCR

20 µL reaction was used for DNA extraction and purification with a 1X SYBR Premix (Takara Bio Inc, Shiga, Japan), 1µL of the extracted genomic DNA and Universal Primer (F-5'-GATTAGATACCCTGGTAGTCCAC-3',R-5'-TACCTTGTTACGACTT-3'), which were validated priorly using conventional polymerase chain reaction. The quantification of Porphyromonas gingivalis was done using species specific primer (F:5'ACCTTACCCGGGATTGAAATG-3',R:5'CAACCATGCAGCACCTACATAGAA-3') (Amplicon size:83bp). The assay was performed on the Bio-Rad CFX96 thermal cycling system. The following program was followed: 95℃ for 3 minutes, followed by 39 cycles of 95℃ for 10 seconds and 54℃ for 3 minutes annealing temperature. Fluorescence signals were measured every cycle at the end of the extension step. The resulting data was analyzed using CFX Maestro Software, Bio-Rad, California, USA.

Statistical analysis

Statistical analysis was done using the Statistical Package of Social Services Software, Version 23 (SPSS Inc., Chicago, IL, USA). Intra-group analysis was done using the paired t test and the inter-group analysis was carried out using the independent t test.

RESULTS

Total bacterial copy count

At baseline, the total bacterial copy count in group A and group B were $0.26x10^8$ and $0.28x10^8$ respectively. The difference was not statistically significant (p=0.92). After the usage of these two mouthwashes over a period of one month, the post-operative total bacterial copy count in group A and group B were $0.0147x10^8$ and $0.0141x10^8$ respectively. The total bacterial copy count decreased exponentially after using both the mouthwashes, however the results were not statistically significant (p=0.827). (Table 1, Table 2)

Porphyromonas gingivalis copy count

At baseline, the Porphyromonas gingivalis copy count in group A and group B were $0.0027x10^8$ and $0.0028x10^8$ respectively. The difference was not statistically significant (p=0.661). After the usage of both the mouthwashes for a period of one month, the post-operative Porphyromonas gingivalis copy count in group A and group B were $0.0025x10^8$ and $0.0012x10^8$ respectively. It was observed that the Porphyromonas gingivalis count was seen to be significantly lesser in group B (p=0.000). (Table 1, Table 2)

TABLE 1: Comparison of total bacterial and Porphyromonas gingivalis copy count numbers between group A and group B at baseline

	Group A	**Group B**	**p value**
Total bacterial copy count (10^8 copy/ml)	0.26	0.28	0.92
Porphyromonas gingivalis copy count (10^8 copy/ml)	0.0027	0.0028	0.661

TABLE 2: Comparison of total bacterial and Porphyromonas gingivalis copy count numbers between group A and group B at 1 month follow-up

	Group A	**Group B**	**p value**
Total bacterial copy count (10^8 copy/ml)	0.0147	0.0141	0.827
Porphyromonas gingivalis copy count (10^8 copy/ml)	0.0025	0.0012	0.000

DISCUSSION

The present study was done to evaluate the effect of an oxygen enriched mouthwash compared to chlorhexidine on the periodontal microflora by using real time polymerase chain reaction (RT-PCR) in patients undergoing dental implant placement.

The outcome of the present study showed a significant reduction in microbial load in both groups. Chlorhexidine, considered the gold standard amongst the antimicrobial agents, has been found extremely effective as an adjunct to mechanical plaque control.27,28 According to a systematic review of clinical studies performed over a 25 year period, chlorhexidine was seen to have a positive effect on plaque control as well as patients with gingivitis, our results are in accordance with the systematic review.29 However, the main drawbacks of continuous use of chlorhexidine mouthwash include the formation of stains on the surfaces of teeth, fillings, oral mucosa and even dental prosthesis, an increase in calculus formation, and altered taste perception.30,31 These drawbacks can be countered by exploring various other mouthwashes available that bypass these limitations.

An alternative agent, using sustained oxygen release to combat gram negative subgingival microflora has now emerged, the oxygen enriched mouthwash which was formulated to counter the disadvantages faced with chlorhexidine use. Sodium perborate is used as an active agent and when it comes in contact with water, it causes hydrolysis releasing hydrogen peroxide and boric acid. Hydrogen peroxide then further reacts with saline and releases nascent oxygen. Lower concentrations (<0.15%) of hydrogen peroxide does not cause tissue damage to healthy host cells and aids in the lysis of pathogenic bacteria.32 The results from our study showed that, while both mouthwashes were effective in overall reduction in oral microflora, oxygen enriched mouthwash showed statistically significant differences when compared to chlorhexidine mouthwash in reducing the load of gram negative Porphyromonas gingivalis, a red complex organism with key role in the progression of peri-implantitis as well as periodontitis. This is in accordance to a study done where the comparative evaluation of the microbial load of patients with chronic periodontitis undergoing scaling and root planing, oxygen enriched mouthwash and chlorhexidine mouthwash was used and found to be of comparable effectiveness.26

The rising complication of placing dental implants is peri-implantitis. Peri-implantitis is the inflammation of the surrounding soft and hard tissues leading to subsequent failure of the dental implant. The cause of peri-implantitis is multifactorial, but an increase in the microbial load due to the formation of a plaque biofilm is an important factor. Therefore, the use of such antimicrobial mouthwashes that deliver the drug to the subgingival regions and help combat the build up of gram negative bacteria is of key importance. Maintenance after implant placement and stringent recall practices along with powerful adjuncts can help prevent adverse complications like peri-implantitis.

CONCLUSION

Oxygen enriched mouthwash can be used as a safe alternative to chlorhexidine in reducing the microbial load during the post operative healing period following dental implant placement.

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