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Novel Peroxide Peroxide Gel for Management of Stage II Periodontitis

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Abstract:

Background:
H_2O_2 is a powerful oxidizing agent with anti-microbial effect that is widely used in dentistry for many purposes. In field of Periodontics, it is used as subgingival irrigant. But the liquid form can damage soft tissues. When it used in the form of gel, its flow is reduced enhancing its antimicrobial property without damage to oral soft tissues. The aim of the study was to evaluate the clinical & microbiological efficacy of novel peroxide gel along with mechanical therapy for Stage II Periodontitis subjects.

Materials and methods:
A total of 80 patients were recruited for the study and were divided into two groups: GROUP I: Patient treated with SRP & novel peroxide gel (n= 40) GROUP II: Patients treated with SRP alone (n= 40). Plaque Index, Sulcus Bleeding Index, Probing Depth, Clinical Attachment Level were recorded at baseline and 1 month after SRP. Total subgingival flora was counted using Colony forming units at baseline and one month after therapy and *P. gingivalis* count from subgingival plaque at baseline and one month after therapy were assessed using real time-PCR.

Results:
Intragroup comparison of Clinical parameters showed significant difference at baseline and one month after treatment ($p < 0.0001$, $p < 0.0001$). Intergroup comparison of clinical parameters showed significant difference in Group I after one month ($p < 0.0001$) than Group II. Intergroup comparison of Microbial analysis [CFU, PCR analysis] showed significant reduction in group I compared to group II ($p < 0.0001$) one month after treatment.

Conclusion:
Though SRP is a hallmark treatment, it cannot completely control disease progression. Wonder gel has antimicrobial efficacy that could be used effectively as an adjunct to SRP in patients with Stage II Periodontitis.

Key words: Colony Forming Units, Hydrogen Peroxide, *P. gingivalis*, Periodontitis, RT- PCR, SRP.

INTRODUCTION:

Periodontitis is an inflammatory disease caused by pathogenic microorganisms in dental plaque resulting in periodontal pocket formation, loss of attachment and alveolar bone around the tooth.[1] Scaling and Root Planing [SRP] is a gold-standard treatment for Periodontitis that is performed to reduce the subgingival microbial load and disrupt microbial biofilm. SRP can be supplemented with subgingival irrigation, systemic antibiotics or local drug delivery to improve the treatment outcome. [2] History of hydrogen peroxide usage in dentistry dates back to more than 70 years. Hydrogen peroxide has been utilized in early 1930s to control dental plaque formation.[3] H_2O_2 effectively kills bacteria through radical generation without bacterial resistance because of the non specific oxidative damage to cell structure. H_2O_2 has one unpaired electron in its structure that destabilizes easily creating free-radicals which has oxidizing potential [4]. H_2O_2, when exposed to light, releases hydroxyl radicals that lead to microbial damage by reacting with extracellular matrix.

Hydroxyl radicals possess very short life in liquid medium [5]. The yield of OH^- radicals from hydrogen peroxide additively increases by homolytic fission when irradiated with visible light which is known to be **photolysis**.[6] Exposure of the oral mucosa to hydroxyl radicals generated by H_2O_2 photolysis for a short time does not cause lethal histological changes.[7] Based on the limitations of liquid H_2O_2 in previous studies, formulation of H_2O_2 in a gel form was attempted. Thus the aim of the study was to evaluate the clinical and microbiological efficacy of Novel peroxide gel with SRP for management of Stage II Periodontitis subjects.

MATERIALS AND METHODS:

This study was designed and conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013, in the Department of Periodontics & Implantology, Thai Moogambigai Dental College & hospital, Chennai. Ethical clearance for the study (Ref: Dr. MGRDU/TMDCH/2018-19/1881801) was obtained from the Institutional Ethics Committee, Dr. MGR University & research institute, Chennai. Preparation of peroxide gel was carried out at Faculty of Pharmacy. Microbiological assay was carried out at the Department of Microbiology, ACS Medical College & Hospital.

STUDY POPULATION:

The present study was conducted for a period of 2 month (3rd September 2018 – 3rd November 2018). A total of 100 Periodontitis patients who came to the Department of Periodontics & Implantology were examined, out of which 80 patients (41 females and 39 males) meeting the selection criteria were randomly selected. Patients within the age group of 20 - 45 years, with a minimum of 20 natural teeth, periodontitis Stage II (PPD ≥ 5mm) Grade A & requiring non-surgical periodontal therapy were recruited for the study. All the participants of the study were explained about the study protocol and an informed written consent was obtained. All participants promptly reported for the follow up appointments. No patient in Group I reported with allergy or burning sensation to the gel. Participants were examined thoroughly and were randomly divided into two groups:

Group I: 40 patients were treated with SRP followed by application of novel peroxide gel

Group II: 40 patients were treated with SRP alone.

Pregnant women, lactating mothers, smokers, patients with systemic diseases, known allergy to hydrogen peroxide, patients who were under medications (antibiotics or NSAID's) for past 6 months were excluded from the study. Clinical parameters namely Plaque Index (Silness & Loe 1964), Sulcus Bleeding Index (Muhlemann & son 1971) Periodontal Probing Depth, Clinical Attachment Level were documented at baseline & one month after treatment. Subgingival plaque samples were collected from all the participants at baseline & one month after treatment for quantification of total bacterial count using CFU and *P. gingivalis* using real time PCR.

PREPARATION OF GEL:

The preparation of gel was done at the Faculty of Pharmacy, ACS Medical College & hospital, Chennai. For peroxide gel, 3% hydrogen peroxide was prepared in the pharmaceutics laboratory by dissolving 30% Hydrogen peroxide in 100 ml of distilled water. Polaxamer (188) Grade A was added to the prepared hydrogen peroxide. This was mixed using a magnetic stirrer for 2 hours. Methyl paraben 0.05% was added to the final product as preservative and transferred to the syringe [Fig 1]. The gel had reverse hydrocolloid property due to polaxamer which can turn sol into gel when exposed to room temperature [Fig 2]. The gel was stored in refrigerator at 4 ºC and used within a week due to short life span of hydrogen peroxide. The prepared gel was subjected to laboratory analysis to confirm the percentage of hydrogen peroxide.

Methodology:

The patients were randomly selected in the study by lottery method. Clinical parameters were recorded at the baseline and 30 days after the treatment. A thorough full mouth Sub-gingival scaling and root Planing were performed for all 80 patients by two clinicians using ultrasonic scaler and Gracey's curettes (Hu- fridey). The gel was placed inside the deepest pocket (5mm) in Group I. Visible blue light of 450 nm wavelength [composite resin curing light unit] was applied in the area where gel was placed and cured for 3 minutes [Fig 3]. The patients were asked not to eat anything for 1 hour after applying the gel or to brush in that particular area. Patients were refrained from brushing, eating and drinking two hours before plaque sample collection on day 0 and day 30. The patients were given oral hygiene instructions and were asked to brush twice daily using soft toothbrush & toothpaste and avoid using dental floss in the gel applied area.

Collection of Plaque sample

Sub-gingival plaque samples were collected from the deepest pocket at baseline and one month after non-surgical periodontal therapy using sterile Gracey's curette (7/8, 9/10 & 11/12). The plaque samples were immediately transferred to sterile eppendorf tubes containing 50 µl of sterile phosphate buffered saline (pH 7.8). The samples were then transported to the Central Research Laboratory, ACS Medical College & Hospital for quantification of total count using CFU and quantification of *P. gingivalis* using real time PCR. The samples were stored at -20ºC until analysis.

Microbial analysis

The plaque sample was subjected to quantification of microorganisms using Colony forming units. The plaque samples were inoculated in blood agar and incubated at 37 °C in anaerobic condition for 48 hours. The total CFU were calculated using an automated colony counter.

Quantification of *P. gingivalis* in the plaque sample was done using real time PCR. The samples were collected and DNA was isolated. Primer used in the study was PG13. The sequence used was CATCGGTAGTTGCTAACAGTTTTCGPGATGACGTC AAATCAGCACGGCCCTTAC AT. The reactions were carried out in a PE 7700 thermocycler, and the

fluorescence was monitored throughout the reaction. DNA denaturation of samples were done by adding plaque sample to water and heating at 100uC for 1 min, followed by reagents. PCR was carried out under the following conditions: initial cycle of denaturation at 94uC for 5 min and 35 cycles of denaturation at 95uC for 30 s, primer annealing at 64uC for 30 s, extension at 72uC for 1 min, and final extension at 72uC for 5 min. Aliquots of 15 ml of PCR reaction mixture were separated on 1.5% agarose gel in the presence of ethidium bromide and visualized using the GelDoc system (Bio-Rad, USA). Number of P. gingivalis was determined in 5 ml of originally extracted genomic DNA samples and calculated from the standard curve generated.

Statistical analysis

Statistical Software Package SPSS version 22 (IBM SPSS Statistics for Windows, version 22.0, IBM Corp.) was used to perform statistical analysis. Inter-group comparison of clinical parameters& microbial analysis at baseline and after 30 days were analyzed using independent t-test and Intra group comparison of clinical parameters (Plaque Index, Bleeding on Probing, Periodontal Probing Depth, Clinical Attachment Level) & microbial analysis were analyzed using Student's paired t-test. A p-value of less-than or equal to 0.05 was considered statistically significant.

RESULTS:

On Intragroup comparison for all the clinical parameters (Plaque Index, Sulcus Bleeding Index, Periodontal Probing Depth, Clinical Attachment Level) at baseline and one month after treatment, there was a statistically significant reduction observed in Group I & Group II patients (p<0.001) [Table 1]. Inter group comparison of clinical parameters showed significant difference after one month in Group I (P<0.001) [Table 2]. Intragroup comparison of CFU and PCR analysis showed significant reduction (P<0.001) in both the groups one month after treatment **(**CT value is inversely proportional to *P. gingivalis)* [fig- 5]**.** Intergroup comparison of CFU and PCR analysis showed significant reduction in Group I, one month after treatment (P<0.001) [fig- 6].

Table 1: Intragroup comparison of clinical parameters at baseline and after one month

Groups	Plaque index Mean ± SD	Bleeding on probing	Pocket depth Mean ± SD	CAL Mean ± SD
Group I	2.12 ± 0.02	2.16 ± 0.05	3.84 ± 0.06	4.13 ± 0.18
At Baseline	0.90 ± 0.04	1.02 ± 0.03	1.87 ± 0.03	2.77 ± 0.09
After one month	<0.005	<0.0001	<0.0001	<0.0001
ρ Value	Sig	Sig	Sig	Sig
Group II	2.11 ± 0.05	2.16 ± 0.04	3.80 ± 0.12	4.14 ± 0.02
At Baseline	1.28 ± 0.04	1.05 ± 0.03	2.41 ± 0.04	3.06 ± 0.02
After one month	<0.0001	<0.0001	<0.0001	<0.0001
ρ Value	Sig	Sig	Sig	Sig

P value of <0.05 is considered significant

Table 2: Intergroup comparison of clinical parameters after one month

Clinical parameters	Groups	Mean ± SD	P value
Plaque index	Group I	0.90 ± 0.04	<0.0001
Mean ± SD	Group II	1.28 ± 0.04	Sig
Bleeding on probing	Group I	1.02 ± 0.03	0.006
	Group II	1.05 ± 0.03	Sig
Pocket depth	Group I	1.87 ± 0.03	<0.0001
Mean ± SD	Group II	2.41 ± 0.04	Sig
CAL	Group I	2.77 ± 0.09	<0.0001
Mean ± SD	Group II	3.06 ± 0.02	Sig

P value of <0.05 is considered significant

Table 3: Intragroup comparison of microbial analysis at baseline and after one month

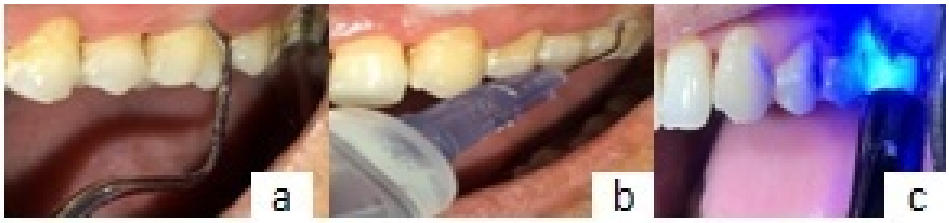
Groups	CT		ρ Value	CFU		ρ Value
	At Baseline	After one month		At Baseline	After one month	
Group I	13.25±0.32	24.23±0.05	<0.0001 Sig	46.42±6.22	8.2±1.42	<0.0001 Sig
Group II	13.27±0.33	18.11±0.06	<0.0001 Sig	45.13±6.46	9.7±1.69	<0.0001 Sig

Table 4: Intergroup comparison of microbial analysis at baseline and after one month

	Group I	Group II	ρ Value
At Baseline (CT)	13.25±0.32	13.27±0.33	0.81645 NS
After one month (CT)	24.23±0.05	18.11±0.06	<0.0001 Sig
At Baseline (CFU)	46.42±6.22	45.13±6.46	0.5341 NS
After one month (CFU)	8.2±1.42	9.7±1.69	0.005 Sig

Figure 1: Peroxide gel stored in syringe

Figure 2: sol to gel conversion



a. Non surgical periodontal therapy performed
b. Placement of peroxide gel inside periodontal pocket in 26
c. Activation of hydrogen peroxide in the periodontal pocket by application of visible light

Figure 3:

DISCUSSION:

Non surgical periodontal therapy has shown to reduce Periodontal Probing Depth (PD) and improve Clinical Attachment Levels (CAL) in mild to moderate periodontitis cases with PD of less than 6 mm [8]. But SRP alone cannot successfully eliminate the microbial load in the periodontal pocket. Use of local drug delivery system along with SRP could enhance the outcome. Hydrogen peroxide along with SRP has proven to be effective in reducing the periodontal pocket depth and improving the clinical attachment level. Photolysis refers to splitting of molecules by the action of light. When hydrogen peroxide is exposed to visible light, it results in formation of 2 HO^- radicals that causes oxidative damage to the subgingival bacteria [4]. H_2O_2 provides a positive pressure similar to hyperbaric oxygen therapy that creates an unfavorable environment to subgingival microflora as most of them are anaerobic bacteria. In our study, hydrogen peroxide was formulated as gel based on limitations of previous studies [9]. Intragroup comparison showed a significant difference in clinical parameters & microbial quantification in both the groups 1 month after SRP from baseline. Significant reduction in clinical parameters was observed in Group I than in group II which was due to the wonder gel placed in Group I patients. Similar result was observed by Putt MS and Proskin HM [9](2010)**,** who locally administered 1.7% hydrogen peroxide gel, using prescription customized trays in moderate to advanced periodontitis and found significant reduction in probing depth and improvement in clinical attachment level. While changes in clinical parameters for group II participants was solely due to the effect of SRP, similar to the study done by Rahul S Bhansali [10] (2014). *P. gingivalis* is a keystone pathogen & is found to be increased in periodontitis patients based on the severity. Gingipains is the main virulence factor possessed by *P. gingivalis.* Previous studies have shown that *P. gingivalis* were resistant to damage caused by oxidative stress but were susceptible to oxidative damage with increased concentration of H_2O_2. This could explain the reason for significant reduction in the level of *P. gingivalis* in Group I [11]. Keller and Buechel [12] (2016) demonstrated a significant change in the periodontal pathogens when treated with direct medication delivery of hydrogen peroxide gel and Vibramycin. Hydroxyl radicals generated by photolysis of H_2O_2 do not diffuse over long distances therefore preventing damage to the adjacent normal unaffected tissues. The bactericidal effect via lethal oxidative damage, such as DNA oxidation and lipid

peroxidation, is exerted only when H_2O_2 is irradiated by visible light. The oxidizing environment created by photolysis enhances the ability of S. sanguis to produce H_2O_2 that affects *P. gingivalis* [13]. Mahdi Z et al [14] (2014) suggested that visible blue light in the presence of erythrosine curcumin and hydrogen peroxide was considered as a potential approach of photodynamic therapy to kill many gram-negative periodontal pathogens such as Porphyromonas and Prevotella species contain protohaemin and protoporphyrin, which absorb strongly at wavelengths of 400-450 nm**.** H_2O_2 provides a positive pressure similar to the oxygen concentration used in hyperbaric oxygen treatment, when delivered into the periodontal pocket that dissociates to 5.7x saturation of oxygen [4]. Wolff et al (1982)[15] studied the effect of H_2O_2 on periodontitis and concluded that 3% H_2O_2 was effective in reducing pocket depth of more than 4mm, but there was no effect on bleeding and gingival index. The mechanism of phototoxicity of blue light on periopathogenic bacteria is oxygen dependant that mainly results in the formation of hydroxyl radicals. Visible light (405–750 nm) has been found to be mutagenic and caused metabolic and membrane damage to bacteria such as Escherichia coli. Visible light has inactivated the growth of Porphyromonas gingivalis and Fusobacterium nucleatum without an exogenous photosensitizer. [16] Applying blue light from a halogen lamp in periodontal pocket had a phototoxic effect on periodontal pathogens, F. nucleatum were killed with 60 seconds of light exposure and P. gingivalis with 15 seconds. [17]

The unique feature of our in-situ gel was its conversion from sol to gel inside the periodontal pocket within 30 seconds. This was due to Polaxamer polymer in the gel which provides the property of remaining as solution at low temperature (4ºC) and readily changing into gel when placed at room temperature. (37ºC) The temperature of the oral cavity caused the conversion of solution to gel within seconds. The advantages of using this gel were its liquid consistency that gets converted into gel when temperature increased, easy availability of the components used, less technique sensitive and cost effectiveness. Use of visible blue light in shallow pockets for activation of hydrogen peroxide had significant effect in Group I. Our study was performed on lesser population for a short period of time which could be a limiting factor of the study.

CONCLUSION

Shallow pocket facilitates better maintenance after active periodontal therapy. The effect of H_2O_2 photolysis treatment was found to be beneficial. H_2O_2 under Visible blue light irradiation penetrates the microbial biofilm in the periodontal pocket that has bactericidal effect against biofilm-forming bacteria. The effect of hydrogen peroxide gel not only reduced microbial load, but also showed better reduction in clinical parameters. Within the limitations of the study, the novel peroxide gel containing could be effectively used as an adjunct to SRP. Further studies with larger sample would be beneficial to prove the result of our study.

List of each author's contribution:

Dr. Gomathi.G.D – Conceptulisation, sample collection, analysis of data, drafting the manuscript.
Dr.S. Gopalakrishnan- Conceptulisation, drafting the manuscript, critical revision
Dr. Uma Sudhakar- Drafting the manuscript, critical revision
Dr. Shifa Fathima- sample collection, analysis of data
Dr. S. Nandhakumar- gel preparation
Dr. D. Dhanalakshmi.- gel standardization

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