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Comparative Evaluation of Conventional Photodynamic Therapy with Hydrogen Peroxide (PDT^{plus}) As an Adjunct to Scaling and Root Planning in Chronic Periodontitis Patients: - A Randomized Controlled Clinico-microbiological Study.

Dr. Mithila Mane[1], Dr. Prashanth Shetty[2], Dr. Varsha Jadhav[3], Dr. Nilambari Jadhav[4], Dr. Shrawani Satish Chouhan[5], Faris Mohammad Akram[6], Sana Mohammad Akram[7]

[1]Ph.D (Scholar), Department of Periodontology, Datta Meghe College, Wardha.
[2]Professor, Department of Periodontology, Yogita Dental College and Hospital, Khed.
[3]Dean, Yogita Dental College and Hospital, Khed
[4]Consultant Periodontist (MDS in Periodontology).
[5]Consultant Periodontist (MDS in Periodontology)
[6] B.D.S. (Scholar), Ras Al Khaimah College of Dental Sciences
[7] B.D.S. (Scholar), Ras Al Khaimah College of Dental Sciences

***Corresponding Author:**
Dr. Mithila Mane
Ph.D (Scholar), Department of Periodontology, Datta Meghe College, Wardha.

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Abstract:
Aim of the study: To evaluate and compare the effect of Conventional aPDT (Photosensitizer and laser) with PDT^{PLUS} (Sub-Gingival Oxidative Irrigant, photosensitizer and laser) in treatment of chronic periodontitis patients. ***Methodology:*** An in-vivo study in which microbiological evaluation was done by allocating two groups having 15 subjects in each group with presence of chronic periodontitis. Group A consists of 15 patients which includes the treatment of Scaling and root planning (SRP) along with Conventional aPDT therapy while Group B consists of other 15 patients include treatment SRP with PDT^{PLUS} therapy. A detailed case history and complete periodontal examination was done including a) Pocket probing depth; b) CAL; c) Plaque index; d) Bleeding on probing; e) Periodontal index. Therefore, GCF samples were collected for microbiological evaluation which includes CFU of gram-ve anaerobic bacteria. Thorough scaling and root planning was done in both the groups followed by Photodynamic Therapies. Follow-up of clinical as well as microbiological evaluation was done at 30th and 90th day. ***Result:*** Reduction in periodontopathic bacteria, probing depth and CAL, were clinically significant in chronic periodontitis patients when treated by PDT^{plus}. ***Conclusion:*** The study can be concluded that PDT^{plus} can be a beneficial treatment protocol being less traumatic, non-invasive, quicker, site specific and less time-consuming therapy in comparison to periodontal surgeries.

Keywords: Photodynamic therapy, hydrogen peroxide, PDT^{plus}

Introduction:

Periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth; progressive attachment loss and bone loss which is because of constant bacterial challenge that alters the subgingival environment. [(1)] So, the therapeutic approach in treating the disease should be elimination of the bacterial plaque. Scaling and root planning is considered as the gold standard for treating periodontal disease which leads to mechanical debridement of plaque and calculus.[(2)] Even though SRP is considered as gold standard it can be impaired sometimes like where there is difficulty in gaining access to the deeper areas.[(3)] The age-old therapies to overcome the persistent disease activity include prescription of antibiotics needed in higher concentrations, which leads to development of bacterial resistance.[(4)] So, to overcome these disadvantages novel modalities were introduced like local drug delivery, photodynamic therapies, etc. Nowadays, photodynamic therapy is gaining a lot of clinical attention because it reduces the drawbacks of Local Drug Delivery (LDD) like not acting on tissue penetrating bacteria and it is also a time-consuming procedure. Recent advances in PDT were introduced like using combination of subgingival irrigant (like Chlorhexidine, (NaCl), Methylpyridinium Chloride, NaOCl, Poly-hexamethylene Biguanide, Stannous Fluoride, Poly-vinyl-pyrrolidone Iodine, H2O2 etc.,) along with conventional aPDT.[(2)]

Photodynamic therapy (PDT) is a laser initiated photochemical reaction, involving the use of a photoactive dye (photo-sensitizer) which is activated by light of a specific wavelength in the presence of oxygen. [(5)] Photodynamic therapy (PDT) was first introduced as a medical therapeutic strategy around 100 years ago to treat cancer, precancerous lesions of face and scalp etc. The term photodynamic therapy was coined by *Jodlbaner* and *von Tappeiner* in 1904 to describe the oxygen-dependent chemical reactions induced by photosensitization. It has emerged in recent years as a non-invasive therapeutic modality for the treatment of various infections by bacteria, fungi, and viruses.[(6)] Thus, the available knowledge of photodynamic therapy should be encouraged for its application in oral diseases like periodontitis, as bacterial plaque is a primary etiological agent.[(7)] PDT involves three components: photo-sensitizer, light and endogenous oxygen and thus in this study we are using Chloride form Toluidine Blue Dye (TBO) as photo-sensitizer, diode laser with 660nm as the light and endogenous oxygen liberated within the pigmented bacteria.

Photo-sensitizer: Antimicrobial photo sensitizers such as porphyrins, phthalocyanines and phenothiazines (e.g., methylene blue and toluidine blue O) have been reported to penetrate into gram-positive and gram-negative bacteria. The positive charge seems to promote the binding of the photo-sensitizer to the gram-negative bacterial membrane and leads to its localized damage, resulting in an increase in its permeability. Hence, toluidine blue O and methylene blue are commonly used in a PDT. [(4)] It is seen that the light band wavelength stimulates a threefold decrease in the growth of *Prevotella melaninogenica, P. nigrescens, P. intermedia, and Porphyromonas gingivalis (Pg)* in the samples of dental plaque collected from the patients with chronic periodontitis. Based on these findings, PDT would slowly decrease the numbers

of black-pigmented bacteria, which results in a healthy environment from a pathological environment. [8] Hence, toluidine blue O and methylene blue are commonly used in a PDT.

Laser: Laser is an acronym for "Light Amplification by Stimulated Emission of Radiation." Lasers function by stimulating the emission of light energy from a given medium in a collimated, focused monochromatic ray of light. The energy beam reacts with a target tissue by being absorbed, reflected, or scattered depending on wavelength and absorption characteristics. Each laser has a unique wavelength spectrum resulting in specific absorption characteristics that must be understood and appreciated for the desired purpose. When applied to the various periodontal tissues such as the gingiva, periodontal ligament, cementum, dentin, and bone, the biologic interactions will be unique for that wavelength. [5] The diode laser uses an electrically pumped semiconductor to produce a laser beam with wavelengths in the range of 655 to 980 nm. Tissue penetration ranges from approximately 0.5 to 5 mm. The laser beam is delivered through a fibre tip. [7]

When a photo-sensitizer is irradiated with light of specific wavelength it undergoes a transition from a low-energy ground state to an excited singlet state. Subsequently, the photo-sensitizer may decay back to its ground state, with emission of fluorescence, or may undergo a transition to a higher-energy triplet state. The triplet state can react with endogenous oxygen to produce singlet oxygen and other radical species, causing a rapid and selective destruction of the target tissue. This utilization of oxygen in the production of reactive oxygen species is known as photochemical oxygen consumption. The triplet-state photo-sensitizer reacts with biomolecules by two mechanisms. [6]

Type I: It involves electron/hydrogen transfer directly from the photo-sensitizer, producing ions, or electron/hydrogen removal from a substrate molecule to form free radicals. These radicals react rapidly with oxygen, resulting in the production of highly reactive oxygen species.

Type II: In type II reaction, the triplet state photo-sensitizer reacts with oxygen to produce an electronically excited and highly reactive state of oxygen, known as singlet oxygen which can

interact with many biological substrates inducing oxidative damage on the cell membrane and cell wall. Hence, the reaction takes place within a limited space, leading to a localized response, thus making it ideal for application to localized sites without affecting distant cells or organs. [7]

The new modification of PDT known as the Modified Photodynamic therapy (PDT^{PLUS}) introduced which consists use of subgingival irrigant prior to the conventional aPDT procedure.as per the studies conducted so far, the different sub-gingival irrigants like chlorhexidine, Chlorhexidine, (NaCl), Cetyl-pyridinium Chloride, NaOCl, Poly-hexamethylene Biguanide, Stannous Fluoride, Poly-vinyl-pyrrolidone Iodine, H2O2 etc., have been used only in In-vitro conditions. [2]

Oxygen Irrigant: Sub-gingival irrigation using an antimicrobial agent following Scaling Root Planning (SRP) is based on the assumption that the remaining bacteria after mechanical

debridement can be destroyed this way. Hydrogen peroxide has been used in dentistry in combination with salts or alone for over 70 years. Therapeutic delivery of H2O2 to prevent or treat periodontal disease, requires mechanical access to subgingival pockets. Furthermore, it enhances the antimicrobial effect of the therapy due to topically administered hydrogen peroxide.

Hydrogen peroxide has shown to possess a wide spectrum of antimicrobial activity in that it is active against bacteria, yeasts, fungi, viruses and spores. The nonsurgical periodontal therapy combining SRP with hydrogen peroxide is gaining more and more consensus in clinical dental practice as shown in the Rey protocol given by ***Dr. Gerard Ray in 2016.*** [(9)]

As the use of H_2O_2 is having an additive effect than conventional SRP, in the present study we have chosen the combination of using H_2O_2 as Sub-gingival irrigant along with conventional aPDT that is PDT^{PLUS}. The benefits of hydrogen peroxide as opposed to classic photodynamic therapy (PDT) performed with photo-activating agents with absorption within the visible band, consist in a higher bioavailability and deeper penetration in the biofilms as well as in the scarce interference in respect to the irradiation performed. [(10)]

Therefore, next to conventional PDT, a new modified PDT^{plus} procedure with improved antibacterial efficacy was implemented. While there are numerous studies investigating the antimicrobial efficacy of different subgingival irrigation and PDT separately, only a few studies are available comparing both methods either as a single application or in combination. It is an interesting approach to know the additive antimicrobial effect of both H_2O_2 along with PDT in periodontal therapy. [(2)]

Materials and methods:

The present study was a clinical and microbiological study. The study protocol was approved by the ethical committee of Yogita Dental College and Hospital, Khed. The sample size of 30 patients was finalized. Prior approval for the study was obtained from the local ethical committee. The patients were explained regarding the study procedure and written consent was obtained from those who agreed to participate voluntarily in this study. These patients were divided into following 2 groups:

Group A [15 patients] – Scaling and root planning along with conventional photodynamic therapy (i.e. with Laser and TBO) and
Group B [15 patients] – Scaling and root planning along with photodynamic plus therapy (i.e. with Laser, Toluidine Blue Dye and Hydrogen Peroxide).

Inclusion Criteria:

1. Patients diagnosed with chronic periodontitis.

2. Individuals with age greater than 25 years and have no risk factors for periodontitis.

3. Full-mouth plaque and bleeding scores ≤15%, probing depths>3 mm and /or CAL of ≥ 3mm at 30% or more of the present teeth.

4. Systemically healthy individuals.

Exclusion Criteria:

1. Pregnancy or current intention to become pregnant, as well as lactating women.

2. Participation in another clinical trial.

3. Any kind of medication that could interfere with the periodontal tissue health or healing.

4. Contraindication for periodontal therapy.

5. Wearing orthodontic appliance/pulp pathology /mal positioned.

6. Individuals with known systemic diseases.

7. Patients who are smokers, alcoholics and having any other adverse habits.

8. Photosensitivity disorders and allergy to Toluidine blue dye.

Withdrawal Criteria: 1. Failure to report for revaluation.

2. Non-Compliant Subjects.

Fig. 01:- Armamentarium for GCF Collection, Scaling Root Planing and Photodynamic Therapies

Methodology:

A. At Baseline: A detailed medical and dental case history was recorded followed by the complete periodontal examination with following indices were done using manual calibrated periodontal probe (UNC-15) which included:

a) Pocket probing depth;

b) Clinical attachment loss;

c) Plaque index (Loe and Sillness, 1964);

d) Bleeding on probing [Muhlemann H.R & Sons-1971] [SBI];

e) Periodontal index [RUSSELL,1956].

GCF samples for microbiological evaluation were collected using paper strips and transported within 48 hrs. via RTF medium to the laboratory for analyzing colony forming units of gram-negative anaerobic bacteria.
Thorough scaling and root planning was done in both the groups followed by Photodynamic Therapies.

B. Photodynamic Therapies

Group A - Conventional Photodynamic Therapy

In the control group, patients treated with scaling and root planning were immediately treated using TBO and Laser Radiations only. Periodontal pockets were irrigated with TBO for 60s followed by application of laser for 60secs. After the PDT procedure is completed, patients were recalled for a follow up after 30 and 90 days and were evaluated for clinico-microbiological parameters.

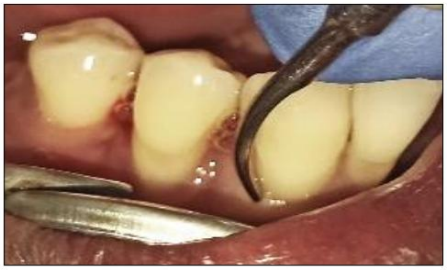


Fig. 03:- Scaling and Root Planing

Group B- Hydrogen Peroxide Supplemented Photodynamic plus Therapy.

In the test group, patients treated with scaling and root planning were administered with 3% H2O2 supplemented with TBO dye along with laser radiation. In the PDT^{plus} therapy approach, 3% hydrogen peroxide solution was irrigated in periodontal pockets immediately followed by application of freshly prepared TBO dye in pockets using syringe for 30-60s. The further proceedings of PDT^{plus} will correspond to the conventional PDT as described in the previous paragraph (60 sec exposure to TBO, 60 sec irradiations with laser radiation) after the therapy, patients were recalled for follow up after 30 and 90 days and were evaluated for clinico-microbiological parameters.

C. Collection of GCF Samples:

GCF collection was performed at the sites by properly inserting sterile Dura pore GCF strips into the gingival crevice until mild resistance is felt. It was then left in place for 30s and proper isolation is maintained to avoid contamination of saliva (Ref Fig. 04). The samples were then carried to the microbiological lab immediately through a Reduced Transport Fluid medium analysing colony forming units of gram-negative anaerobic bacteria (Fig. 05).

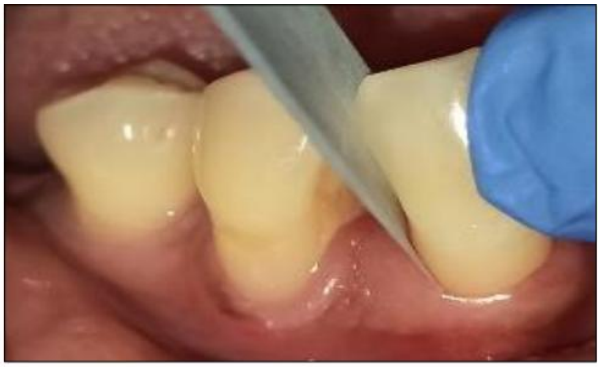


Fig. 04:- GCF Collection for Microbial Evaluation

Fig. 05:- Eppendorf Tube Containing Reduced Transfer Medium Along With Sample

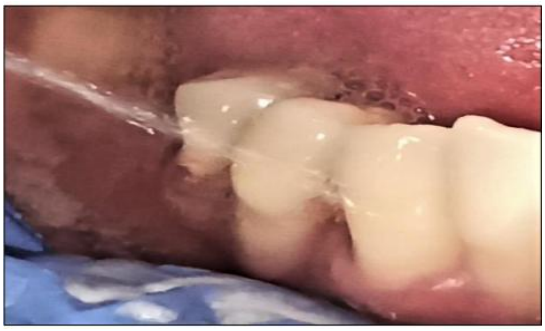


Fig. 06: - Application of 3% Hydrogen Peroxide as the Oxygen Agent

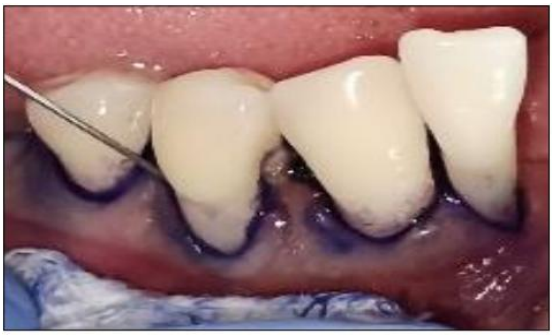


Fig. 07:- Application of Toluidine Blue O Stain

Fig. 08:- Application of Diode Laser Therapy

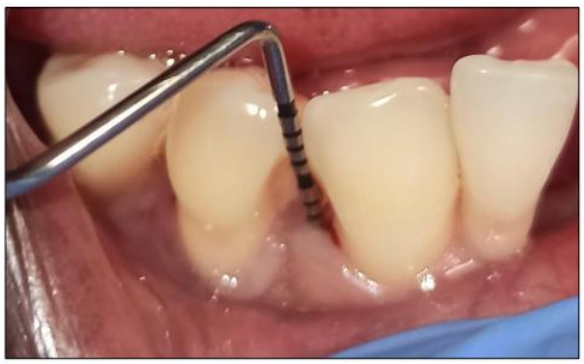


Fig. 02:- Pocket Probing Depth Recorded After One Month

D. Follow Up and Evaluations:

The patients from both the groups were followed up and evaluated for Clinico-microbiological examinations at Baseline, 30th day and 90th day.

MICROBIAL CULTURE PROCEDURE

Samples were sent in the Reduced Transport Fluid Medium (RTF) & were first vortexed then they were inoculated in the culture medium according to the requirement in Enriched and Selective medium. They were cultured anaerobically for 72 Hrs. at 37°C on Blood Agar. After 72 Hrs. growth of bacterial colonies were seen on culture plates. Blood Agar was used as an enriched medium along with Brucella Agar (BA), Hemin and Vitamin K as the selective medium. One was incubated aerobically and the other was incubated anaerobically in an anaerobic jar. Then after completion of incubation, the plates were removed, and the colony characters of the required organism were noted along with the colony count for quantification. Bacterial colonies of Group A and Group B were serially diluted at different ratios in Brucella Agar, Hemin and sub-cultured under the same cultural conditions followed by incubation for 72 Hrs. After incubation, the number of viable bacterial counts was evaluated.

Fig. 09:- Microbial Culture of **Control Group before** Treatment

Fig. 10:- Microbial Culture of **Control Group after** Treatment

Fig. 11:- Microbial Culture of **Test Group before** Treatment

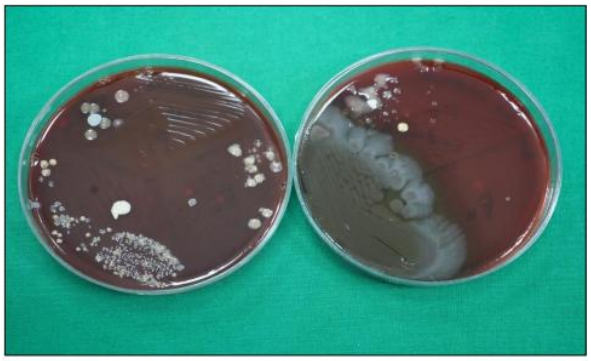


Fig. 12:- Microbial Culture of **Test Group after** Treatment

Statistical Analysis

All the data were entered into Microsoft Excel 2010. Descriptive statistics were expressed as mean ± standard deviation (SD) for each group for various clinical parameters. For Age Frequency distribution and percentage were used. Discrepancy among groups and age distribution was calculated by Chi square Test. Discrepancy among groups and gender distribution was calculated by Fishers' Exact Test.

Baseline, after 30 days, after 90 days comparison among all groups for all clinical parameters was done by Repeated Measures Analysis of Variance Followed by Bonferroni's post hoc test for pairwise comparison. Two groups (Control group versus Test Group) were compared for various parameters by Unpaired 't' Test (Independent 't' test)

For All the above test p value is considered statistically significant when it was <0.05. The software used was SPSS (Statistical package for Social Sciences) version 17.

Results: **Table 1 Age Statistics among two groups**

groups	N	Minimum	Maximum	Mean	Std. Deviation	**Fisher Exact Test p value**
Control Group	15	39	63	51.00	7.792	.546*
Test Group	15	28	65	48.93	9.750	

The mean age among the Control Group **was** 51.00 ± 7.792, among the Test Group it was 48.93± 9.750. There is statistically insignificant difference among two groups with p=0. 546

Chart 1 Age Statistics among Two groups

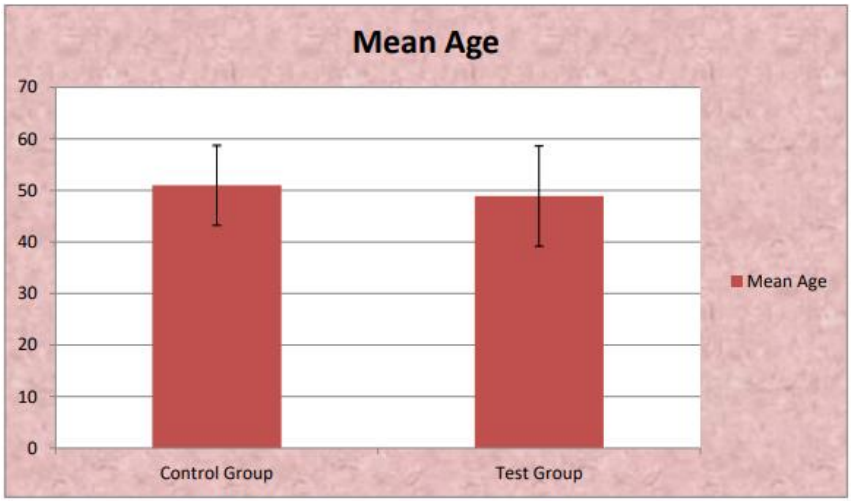


Table 2 Gender Statistics among two groups

Groups		Frequency	Percent	Valid Percent	Cumulative Percent	**Chi square Test P value**
Control Group	Male	11	73.3	73.3	73.3	0.439
	Female	4	26.7	26.7	100.0	
	Total	15	100.0	100.0		
Test Group	Male	9	60.0	60.0	60.0	
	Female	6	40.0	40.0	100.0	
	Total	15	100.0	100.0		

***Statistically Significant**

There is statistically insignificant difference among two groups for gender distribution with p=0.439

Graph 2: Gender Distribution among Two groups

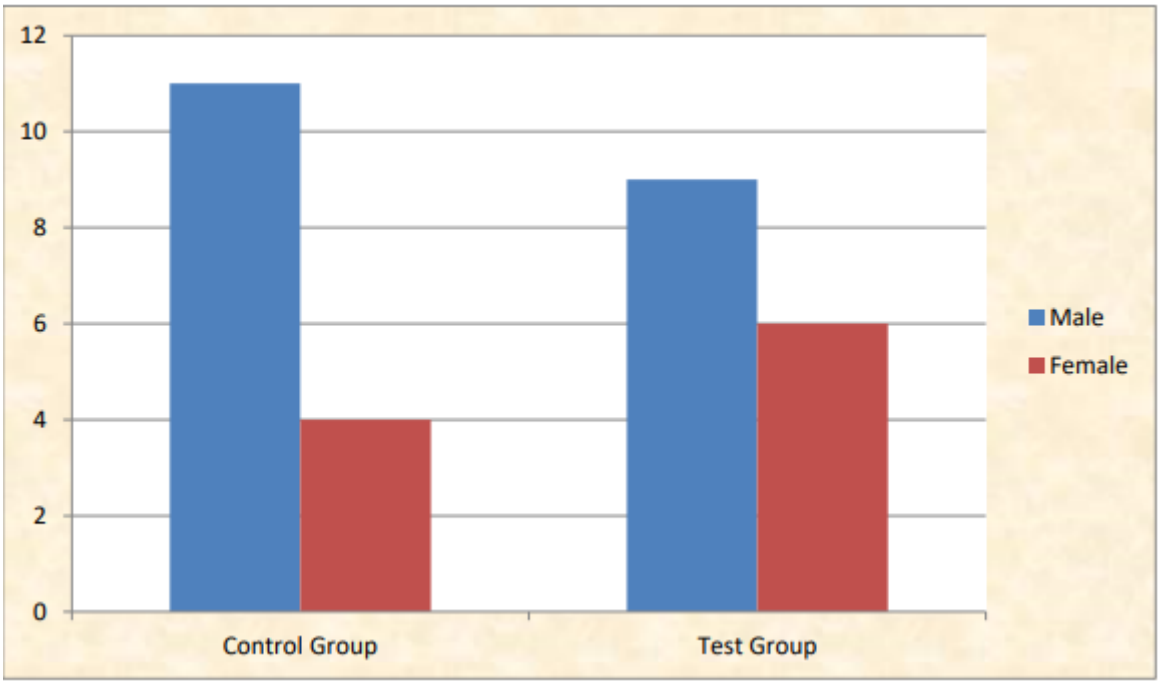


Table 3: Inter and Intra group comparison of Plaque Index (PI) among Control and Test Groups by Unpaired 't' test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test respectively

Plaque Index (PI)	(Control Group) (n=15)		(Test Group) (n=15)		P-value
	Mean	**SD**	**Mean**	**SD**	**(Inter-Group)**
Baseline	2.4827	.47870	2.2467	.56172	0.226
After 30 days	1.5953	.82938	0.9153	0.39366	0.008*
After 90 days	1.0733	.57255	0.7660	0.46567	0.146
P-value (Intra-group) Repeated Measures ANOVA	<0.001*				
Baseline v After 30 days	0.001*		<0.001*		
Baseline v After 90 days	<0.001*		<0.001*		
After 30 days v After 90 days	0.146		1.000		

P-values (Inter-Group) by Independent sample t test. P-value (Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test. P-value<0.05 is considered to be statistically significant. *P-value<0.05, **P-value<0.01, ***P-value<0.001, NS-Statistically non-significant.

(Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test for Plaque Index

Control group has shown statistically significant difference in Plaque Index from baseline to 30 days (p<0.001*) and 90 days (<0.001) but there was no statistically significant difference when compared from 30 days to 90 days (p=0.146)

Test group has shown statistically significant difference in Plaque Index from baseline to 30 days ($p<0.001$*) and 90 days ($p<0.001$*) and there was statistically significant difference when compared from 30 days to 90 days($p=1.000$)

(Inter-Group) by Independent Sample T Test

There is statistically insignificant difference in Plaque Index between both groups at baseline (0.226), at 30 days (0.008) and at 90 days (0.146).

Graph 3: Inter and Intra group comparison of Plaque Index (PI) among Control and Test Groups by Unpaired 't' test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test respectively.

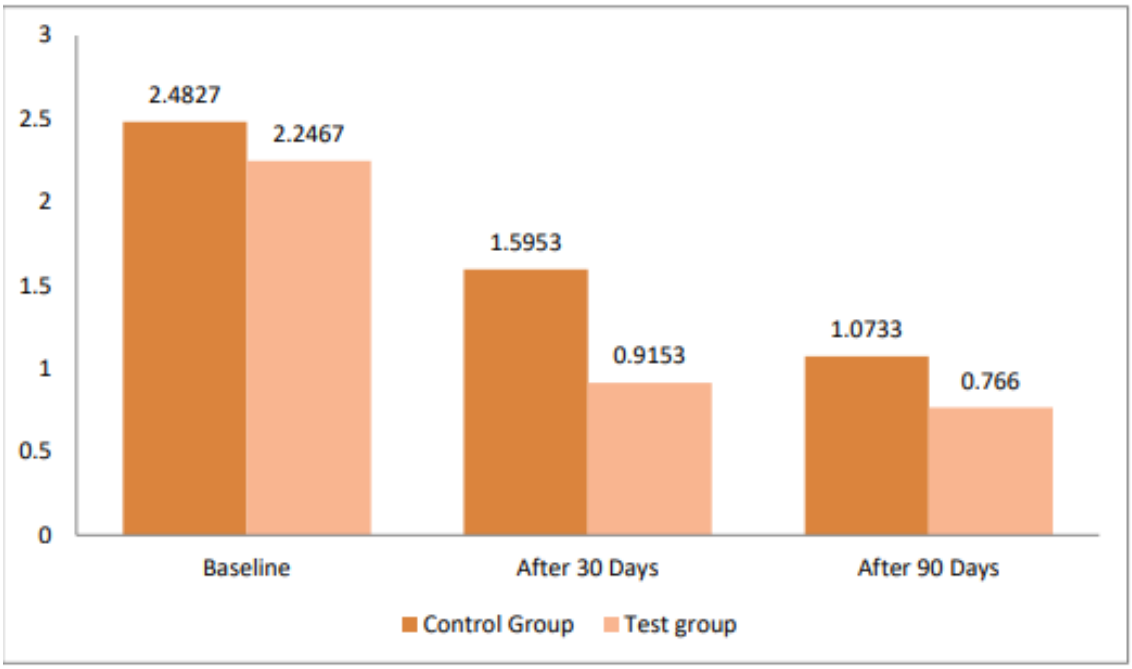


Table 4: Inter and Intra group comparison of Sulcus Bleeding Index (SBI) among Control and Test Groups by Unpaired t test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test respectively

Sulcular Bleeding Index (SBI)	(Control Group) (n=15)		(Test Group) (n=15)		P-value
	Mean	**SD**	**Mean**	**SD**	**(Inter-Group)**
Baseline	3.2600	1.72190	3.5420	.53121	0.233
After 30 days	2.3620	.1.13826	1.6253	0.74936	0.045
After 90 days	.8940	.57019	1.1900	.67618	0.206
P-value (Intra-group) Repeated Measures ANOVA	<0.001*		<0.001*		
Baseline v After 30 days	<0.001*		<0.001*		
Baseline v After 90 days	<0.001*		<0.001*		
After 30 days v After 90 days	<0.001*		<0.001*		
P-values (Inter-Group) by Independent sample t test. P-value (Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test. P-value<0.05 is considered to be statistically significant. *P-value<0.05, **P-value<0.01, ***P-value<0.001, NS-Statistically non-significant.					

(Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test for Sulcus Bleeding Index

Control group has shown statistically significant difference in Sulcus bleeding index from baseline to 30 days ($p<0.001^*$) and 90 days ($p<0.001^*$) and there was also statistically significant difference when compared from 30 days to 90 days ($p<0.001^*$) Test group has shown statistically significant difference in Sulcus Bleeding Index from baseline to 30 days ($p=0.001^*$) and 90 days ($p<0.001^*$) and there was also statistically significant difference when compared from 30 days to 90 days ($p<0.001^*$)

(Inter-Group) by Independent Sample T Test

There is statistically insignificant difference in Sulcus Bleeding Index between both groups at baseline (0.233), at 30 days (0.045) and at 90 days (0.206).

Graph 4: Inter and Intra group comparison of Sulcular Bleeding Index (SBI) among Control and Test Groups by Unpaired t test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test respectively.

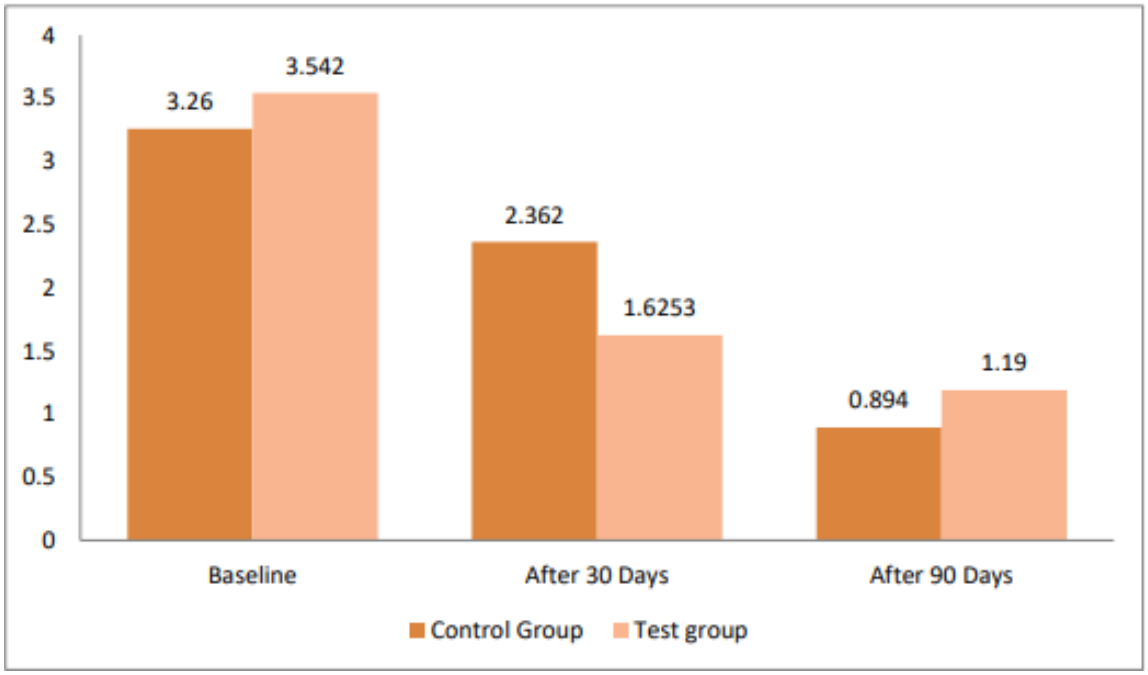


Table. 5 Inter and Intra group comparison of Russell's Periodontal Index (RPI) among Control and Test Groups by Unpaired t test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test respectively.

Russell's Periodontal Index (RPI)	(Control Group) (n=15)		(Test Group) (n=15)		P-value
	Mean	**SD**	**Mean**	**SD**	**(Inter-Group)**
Baseline	3.8133	.62091	5.1800	.64609	<0.001*
After 30 days	2.8627	1.29525	1.7427	67464	0.006*
After 90 days	1.3800	.62701	1.6167	.78414	0.369
P-value (Intra-group) Repeated Measures ANOVA	<0.001*		<0.001*		
Baseline v After 30 days	<0.001*		<0.001*		
Baseline v After 90 days	<0.001*		<0.001*		
After 30 days v After 90 days	0.008		<0.001*		
P-values (Inter-Group) by Independent sample t test. P-value (Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test. P-value<0.05 is considered to be statistically significant. *P-value<0.05, **P-value<0.01, ***P-value<0.001, NS-Statistically non-significant.					

(Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test for Russell's Periodontal Index

Control group has shown statistically significant difference in Russell's Periodontal Index from baseline to 30 days ($p<0.001$) and 90 days ($p<0.001$) but there was no statistically significant difference when compared from 30 days to 90 days ($p=0.008$)

Test group has shown statistically significant difference in Russell's Periodontal Index from baseline to 30 days ($p=0.001$) and 90 days ($p<0.001$) and there was also statistically significant difference when compared from 30 days to 90 days ($p<0.001$)

(Inter-Group) by Independent Sample T Test

There is a statistically insignificant difference in Russell's Periodontal Index between both groups at baseline (p=0.001), but there was no statistically significant difference at 30 days (0.006) and at 90 days (0.369).

Graph 5: Inter and Intra group comparison of Russell's Periodontal Index (RPI) among Control and Test Groups by Unpaired t test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test respectively.

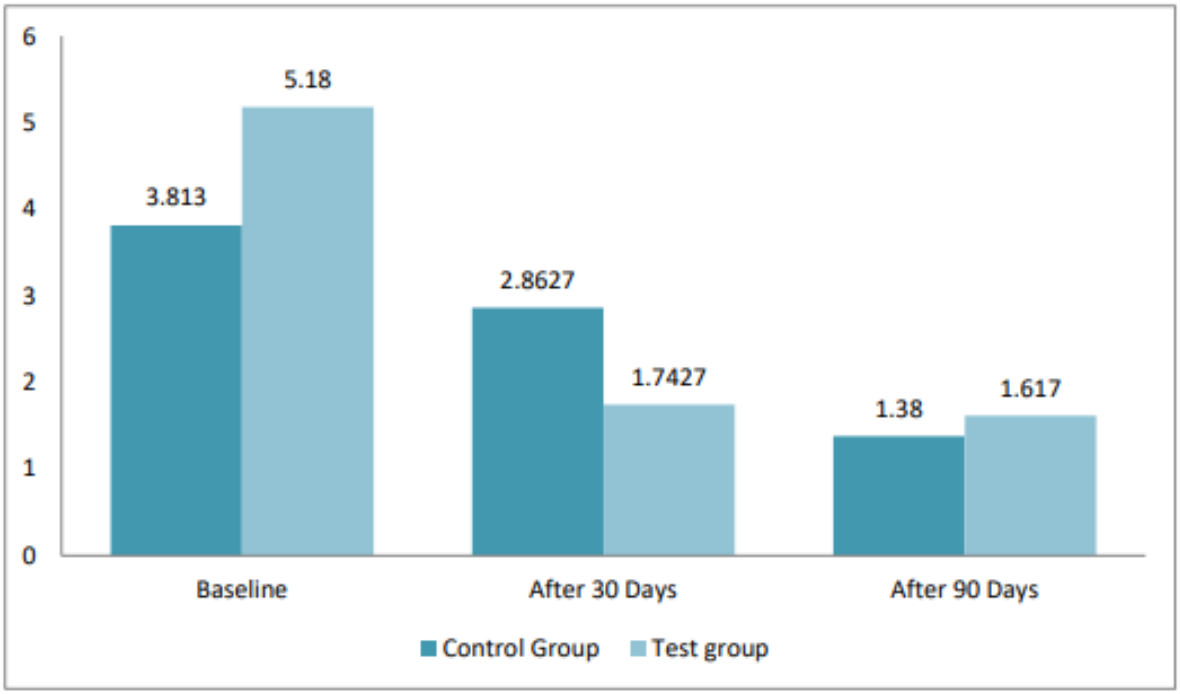


Table 6: Inter and Intra group comparison of Periodontal Pocket Depth (PPD) among Control and Test Groups by Unpaired t test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test respectively

Periodontal Pocket Depth (RPI)	**(Control Group) (n=15)**		**(Test Group) (n=15)**		**P-value**
	Mean	**SD**	**Mean**	**SD**	**(Inter-Group)**
Baseline	6.07	1.100	5.87	.834	0.579
After 30 days	3.80	1.740	3.27	1.033	0.316
After 90 days	2.67	1.047	1.67	0.976	0.011
P-value (Intra-group) Repeated Measures ANOVA	<0.001*		<0.001*		
Baseline v After 30 days	0.004*		<0.001*		
Baseline v After 90 days	<0.001*		<0.001*		
After 30 days v After 90 days	0.089		0.009*		
P-values (Inter-Group) by Independent sample t test. P-value (Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test. P-value<0.05 is considered to be statistically significant. *P-value<0.05, **P-value<0.01, ***P-value<0.001, NS-Statistically non-significant.					

(Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test for Pocket Probing Depth

Control group has shown statistically insignificant difference in Pocket Probing Depth from baseline to 30 days (p=0.004) and 90 days (p<0.001) but there was no statistically significant difference when compared from 30 days to 90 days (p=0.089)

Test group has shown statistically significant difference in Pocket Probing Depth from baseline to 30 days (p<0.001) and 90 days (p<0.001) but there was no statistically significant difference when compared from 30 days to 90 days (p=0.009)

(Inter-Group) by Independent Sample T Test

There was a statistically insignificant difference in Pocket Probing Depth between both groups at baseline (p=0.579), and there was a statistically significant difference at 30 days (0.316) and at 90 days (0.011).

Graph 6 Inter and Intra group comparison of Periodontal Pocket Depth (PPD) among Control and Test Groups by Unpaired t test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test respectively.

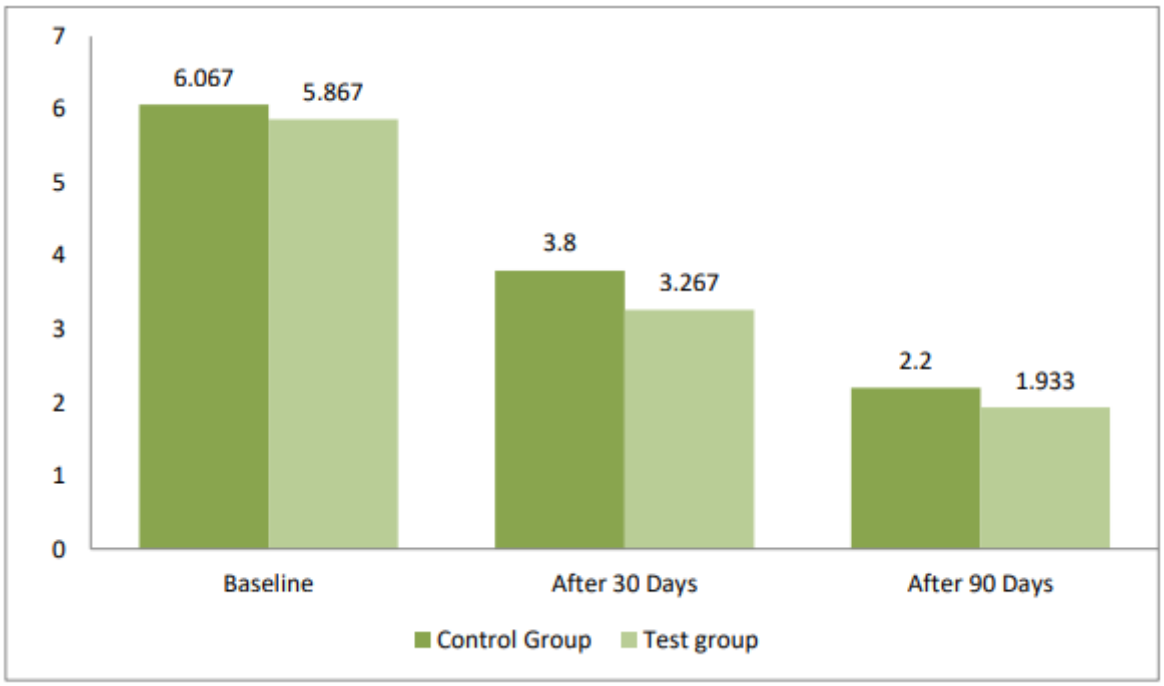


Table 7 Inter and Intra group comparison of Clinical Attachment Loss (CAL) among Control and Test Groups by Unpaired t test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test respectively

Clinical Attachment Loss (CAL)	(Control Group) (n=15)		(Test Group) (n=15)		P-value
	Mean	**SD**	**Mean**	**SD**	**(Inter-Group)**
Baseline	6.60	1.183	5.00	1.414	0.002*
After 30 days	4.07	1.668	2.87	1.552	0.051
After 90 days	2.20	1.146	1.33	1.175	0.050
P-value (Intra-group) Repeated Measures ANOVA	<0.001*		<0.001*		
Baseline v After 30 days	<0.001*		0.001*		
Baseline v After 90 days	<0.001*		<0.001*		
After 30 days v After 90 days	0.001*		0.024*		

P-values (Inter-Group) by Independent sample t test. P-value (Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test. P-value<0.05 is considered to be statistically significant. *P-value<0.05, **P-value<0.01, ***P-value<0.001, NS-Statistically non-significant.

(Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test for Clinical Attachment Levels

Control group has shown statistically insignificant difference Clinical Attachment Levels from baseline to 30 days (p=0.001) and 90 days (p<0.001) and there was statistically significant difference when compared from 30 days to 90 days (p=0.001)

Test group has shown statistically significant difference in Clinical Attachment Levels from baseline to 30 days (p<0.001) and 90 days (p<0.001) but there was no statistically significant difference when compared from 30 days to 90 days (p=0.024)

(Inter-Group) by Independent Sample T Test

There was a statistically significant difference in Clinical Attachment Levels between both groups at baseline (p=0.02), but there was no statistically significant difference at 30 days (0.051) and at 90 days (0.050).

Graph 7 Inter and Intra group comparison of Clinical Attachment Loss (CAL) among Control and Test Groups by Unpaired t test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test respectively.

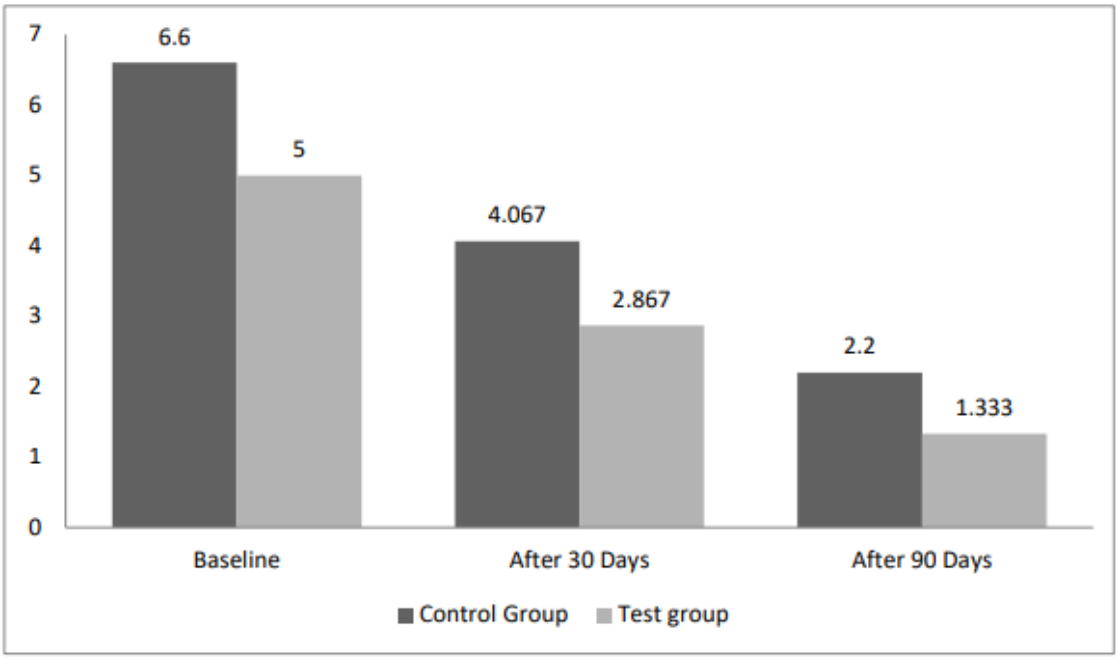


Table 8 Inter and Intra group comparison of Colony Forming Unit (CFU) among Control and Test Groups by Unpaired t test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc test respectively

Colony Forming Unit (CFU)	(Control Group) (n=15)		(Test Group) (n=15)		P-value
	Mean	**SD**	**Mean**	**SD**	**(Inter-Group)**
Baseline	499.33	94.753	468.00	95.708	0.375
After 30 days	231.33	111.667	188.67	72.690	0.225
After 90 days	100.00	55.806	55.33	37.391	0.016*
P-value (Intra-group) Repeated Measures ANOVA	<0.001*		<0.001*		
Baseline v After 30 days	<0.001*		<0.001*		
Baseline v After 90 days	<0.001*		<0.001*		
After 30 days v After 90 days	0.004*		<0.001*		

P-values (Inter-Group) by Independent sample t test. P-value (Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test. P-value<0.05 is considered to be statistically significant. *P-value<0.05, **P-value<0.01, ***P-value<0.001, NS-Statistically non-significant.

(Intra-Group) by Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test for Colony Forming Units

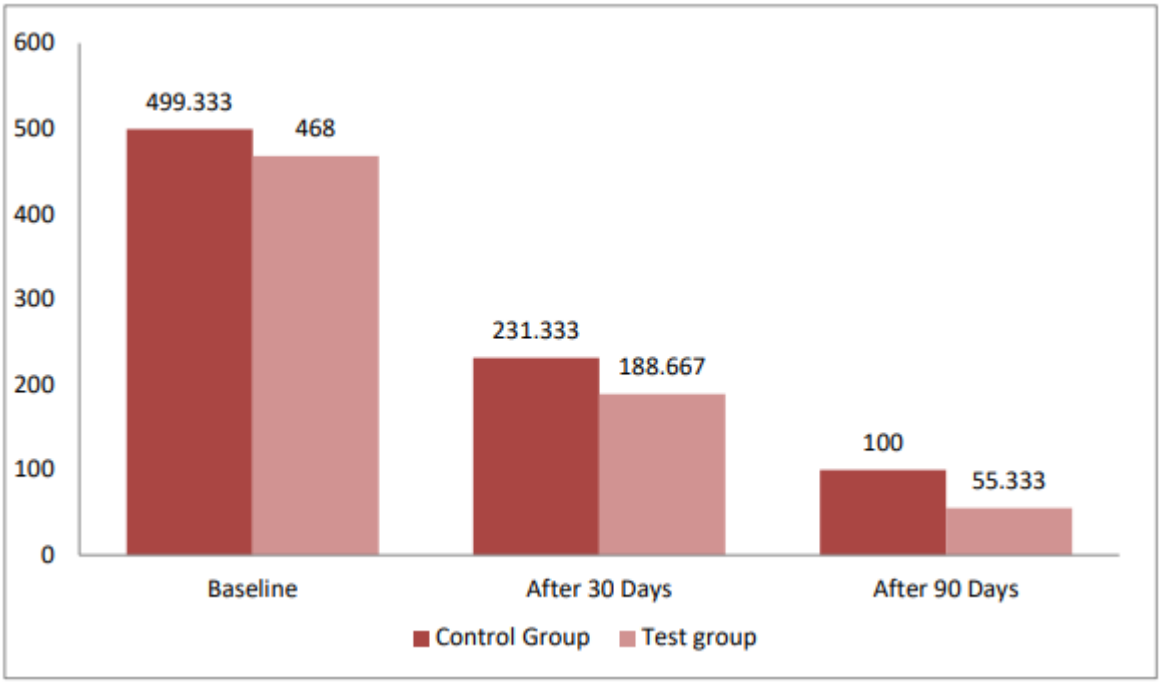
Control group has shown statistically significant difference Colony Forming Units from baseline to 30 days ($p=0.001$) and 90 days ($p<0.001$) but there was no statistically significant difference when compared from 30 days to 90 days ($p=0.004$)

Test group has shown statistically significant difference in Colony Forming Units from baseline to 30 days ($p<0.001$) and 90 days ($p<0.001$) and there was also statistically significant difference when compared from 30 days to 90 days ($p<0.001$)

(Inter-Group) by Independent Sample T Test

There was a statistically significant difference in Colony Forming Units between both groups at baseline (p=0.375) and there was a statistically significant difference at 30 days (0.225) and at 90 days (0.016).

Graph 8 Inter and Intra group comparison of Colony Forming Unit (CFU) among Control and Test Groups by Unpaired t test and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc Test respectively.



Discussion:

Periodontitis is a prevalent disease in the oral cavity caused by gram negative anaerobes which can be treated successfully by mechanical debridement therapy, but in certain patients, the clinical outcome may be impaired by the presence of persistent periodontal pathogens as well as exaggerated host mediated inflammatory response causing progression of the disease. (11) The treatment regimen for such type of cases involves mechanical debridement (SRP) augmented with different non-surgical antimicrobial therapies. Antimicrobial therapies or agents act on tissue penetrating bacteria which are the primary reason for reoccurrence or the persistent periodontal disease, thereby increasing the benefits of when they are used as an adjunct to conventional mechanical debridement. (12)

Recently, one adjunctive therapy has gained clinical attention which is photodynamic therapy and along with that the new modifications of photodynamic therapy have been introduced with the use of oxidative irrigants like Chlorhexidine, NaOCl, Cetylperidium, Sodium Hypochlorite

etc. [13] In our study, we have selected photodynamic therapy and sub-gingival oxidative irrigant H_2O_2 as an adjunct to scaling and root planning. The present study is the only one of its kind, wherein the effect of photodynamic therapy along with oxidative irrigant H2O2 was evaluated clinically as well as microbiologically to relate the outcomes.

Lasers have been advocated for periodontal therapy i.e., for subgingival debridement, reduction of bacterial loads and scaling root planning. [14] ***Aoki et.al.*** did an in-vitro study and concluded that Er: YAG laser does have a potential for clinical application in sub-gingival scaling. Since then, the laser alone was considered as an alternative to overcome the disadvantages of scaling and root planning. [15]

PDT has many advantages such as reducing the treatment time, no need for anaesthesia, destruction of bacteria in a very shorter period and unlikely development of resistance by the target bacteria and it avoids damage to the adjacent host tissues. [16] It basically works on a principle, where a photoactivable substance that binds to the target cell can be activated by light of a suitable wavelength. By irradiation with light in the visible range of the spectrum, the dye is excited to its triplet state and the energy is transferred to molecular oxygen. The product formed is a highly reactive singlet oxygen capable of reacting with biological systems and destroying them. Only the first excited state with energy of 94 kJ/mol (22 kcal/mol) above the ground state is important. [17]

The photosensitizers used for this therapy are applied topically followed by irradiation with the visible light spectrum. Every photoactive dye is activated with a specific wavelength. We chose TBO Dye in our study, since it has many advantages over other dyes that add up to the antibacterial outcomes of PDT. [18] Toluidine Blue O (TBO) is a phenothiazine photo-sensitizer with a cationic charge, which is photo activated by the commonly used wavelength range of 620–680 nm. It has a Broad-Spectrum Antibacterial Activity, with a low mutagenic potential and the ability to eradicate bacterial resistance. In a recent study by ***Parasuraman P. in 2019,*** TBO showed significant photodynamic antimicrobial activity on methicillin resistant S. aureus. [19] It also has some activity on selective cancer cells and has the potential to be developed as an anticancer drug too.

Few studies done by Street C and Pedigo Loebel in 2010[19] have also proved that the association of TBO and diode laser light of 830nm is effective for the killing of bacterial strains and determines the photo inactivation of tissue penetrating microbes i.e., Aggregatibacter actinomycetemcomitans. Thus, it has effectively showed its capabilities in combination with PDT, thereby resulting into use of aPDT.

In conventional antimicrobial therapy, there is increase of bacterial resistance to antibiotics, whereas PDT & PDT^{PLUS} overcome this limitation, using reactive oxygen species to kill bacteria in a short time. [20]

Also, in a study by Moradi et.al. in 2010[9], he concluded from this study that, sub-gingival irrigation with 3% H_2O_2 was very effective in reducing gingival bleeding and inflammation and also in gaining attachment levels, demonstrating a positive clinical effect compared to SRP.

It would be an interesting approach to combine both two methods that would certainly show antibacterial efficiency gaps in periodontal therapies.

In this study we have used the Modified PDT approach (PDT^{plus}), where the photosensitizer TBO was supplemented with 3% hydrogen peroxide irrigation for 30 sec immediately before its use. The application of H_2O_2 as an antiseptic/antibacterial agent followed by the Photo-sensitizer TBO (chlorinated TB) was carried out for 60secs then followed by the laser therapy for 1 min.

The evaluated findings of Plaque, Sulcus Bleeding and Over All Periodontal Status of both Control as well as the Test group showed significant results at baseline, after one month and also after three months in intra-group comparison. But no statistically significant difference was found between the Inter-group results of Conventional PDT and (PDTplus) from 1 month to 3rd month evaluation. The present study results were in concurrence with similar studies done by Qadri et al. [(21)]

In our study, significant gain was found in probing pocket depth (PPD) of both the groups from baseline till intervention after 90 days while the inter- group comparison of control and test group also showed significant results for PPD values with greater reduction in PDT^{PLUS}. This shows that PDT^{plus} is more effective than conventional PDT and these results are coinciding with decreased number of colony forming units of gram-negative bacteria after three months and there are no other current studies evaluating the clinical parameters evaluating the combination yet.

In a study by Cadore et.al. in 2018[(22)], which was a clinico-microbiological randomized controlled trial to assess the efficacy of multiple applications of antimicrobial photodynamic therapy as an adjunct to surgical periodontal therapy from baseline to 90 days. The study concluded significant changes in the microbial species, Clinical Attachment Gain (CAL) and gain in the Pocket Probing Depth (PPD) on multiple sessions of the therapy.

The improvement of adjunctive aPDT in PPD parameter is in line with outcomes of previous clinical studies (Christodoulides et al. 2008, Chondros et al.2009, Braun et al.2008) showed superior results in favour of aPDT in all evaluated parameters. [(23)]

In the microbial profile, a statistically significant change was seen in the CFU count of the gram-negative anaerobic bacteria in the control as well as the test group samples at baseline and at interventions of 30^{th} - 90^{th} day post treatment. On Inter- group comparison test group has shown significant results at 30 days and at 90 days. This shows that H_2O_2 has an additive effect when used with the conventional PDT. This is in accordance to study done by Decker et. al. 2018[(2)] .

Thus, the evaluated findings of the present study suggest that the modified photodynamic therapy PDT^{PLUS} effectively works against the periodonto-pathogens thereby increasing the clinical attachment levels and reducing the probing depths and proving as a beneficial alternative to surgical periodontal therapy for treatment of chronic periodontitis.

Conclusion:

As seen in the present study, the reduction in periodontopathic bacteria, probing depth and clinical attachment levels, were quite clinically significant in chronic periodontitis patients when treated by PDT^{plus} over conventional PDT. PDT^{plus} specifically offered an advantage to overcome the occurrence of species resistance. Also, it needs to be experimented with more sample size with multiple application of H_2O_2 in a long run which may lead to more efficient and significant results. In addition to reducing the clinical parameters in natural teeth as well as peri- implant cases, there are some evidences that PDT will also inactivate virulence factors of periodontal pathogens, enhancing post-treatment outcomes.

Thus, PDT^{plus} can be concluded as a beneficial treatment protocol and less traumatic, non1-invasive, quicker, site specific and less time-consuming therapy in comparison to periodontal surgeries. As an additional new approach, the PDT^{plus} therapy could also be added as an adjunct during the maintenance phases of periodontal therapy. Furthermore, studies experimenting with different aspects of PHOTODYNAMIC THERAPIES with clinically significant objectives, are needed to introduce a greater number of antimicrobial agents, using cost effective supplements along with it.

References:

1. Flemming TF: Periodontitis. Ann Periodontal. 1999; 4:32-38.
2. Decker E-M, Bartha V, Kopunic A, Von Ohle C. Antimicrobial Efficiency of Mouth rinses Versus and In Combination with Different Photodynamic Therapies On Periodontal Pathogens in An Experimental Study. J Periodont Res 2016;52(2):162- 175.
3. Hosaka Y, Saito A, Maeda R Et Al. Antibacterial Activity of Povidone-Iodine against an Artificial Biofilm of Porphyromonas Gingivalis and Fusobacterium Nucleatum. Arch Oral Biol 2012; 57:364–368.
4. Raj K R, Musalaiah S, Nagasri M, Kumar P A, Reddy P I, Greeshma M. Evaluation of Efficacy of Photodynamic Therapy as An Adjunct to Non-Surgical Periodontal Therapy in Treatment of Chronic Periodontitis Patients: A Clinico-Microbiological Study. Indian J Dent Res 2016; 27:483-497.
5. Kumar V, Sinha J, Verma N, Nayan K, Saimbi CS, Tripathi AK. Scope of Photodynamic Therapy in Periodontics. Indian J Dent Res 2015; 26:439-442.
6. Raab O. The Effect of Fluorescent Agents on Infusoria. Z Biol 1900; 39:524-576.

7. Raghavendra M, Koregol A, Bhola S. Photodynamic Therapy: A Targeted Therapy in Periodontics. Australian Dental Journal 2009; 54: S102–S109
8. Nikolaos S. Soukos; J. Max Goodson. Photodynamic therapy in the control of oral biofilms. 2011;55(1):143–166.
9. Moradi Et Al. Clinical Effect of Periodontal Pocket Irrigation with H2O2 DJH 2011;3(1):53-59.
10. Caccianiga G, Baldoni M, Ghisalberti CA, Paiusco A. A Preliminary In Vitro Study on The Efficacy of High-Power Photodynamic Therapy (HLLT): Comparison Between Pulsed Diode Lasers and Superpulsed Diode Lasers and Impact of Hydrogen Peroxide with Controlled Stabilization. Biomed Res Int.2016:1386158.
11. Darby I. Non-Surgical Management of Periodontal Disease. Aust Dent J 2009; 54(1): S86–S95.
12. Feres M, Haffajee AD, Allard K, Som S, Goodson JM, Socransky SS. Antibiotic Resistance of Subgingival Species During and After Antibiotic Therapy. J Clin Periodontal 2002; 29(8):724-735.
13. Petersilka and Flemmig · Periodontal Debridement with Sonic and Ultrasonic Scalers. J Periodontol 2004: 1(4): 353–362.
14. Takamatsu N, Yano K, He T, Umeda M, Ishikawa I, Effect of Initial Periodontal Therapy on The Frequency of Detecting Bacteriodes Forsythus, Porphyromonas Gingivalis and Actinobacillus Actinomycetemcomitans. J Periodontol 1999; 70:574-580.
15. Aoki A, Ando Y, Watanabe H, and Ishikawa I. In-Vitro Studies on Laser Scaling Of Subgingival Calculus with an Erbium: YAG Laser. Journal of Periodontology, 65:1097-1106.
16. Konopka K. Goslinski T. Photodynamic Therapy in Dentistry. J Dent Res .2007;86:694-707.
17. Martinetto P, Gariglio M, Lombard GF, Fiscella B, Boggio F. Bactericidal Effects Induced by Laser Irradiation and Haematoporphyrin Against Gram-Positive and Gram-Negative Microorganisms. Drugs Exp Clin Res 1986; 12:335-342.
18. Moan J, Berg K. The Photodegradation of Porphyrins in Cells That That Can Be Used to Estimate the Lifetime of Singlet Oxygen. Photochem Photobiol 1991; 53:549-53.
19. Parasuraman P. Asha P. Sruthil Lal S. Siddhardha B. Needa A. Turki M. S.; Syed, A. Antimicrobial Photodynamic Activity of Toluidine Blue Encapsulated in Mesoporous Silica Nanoparticles Against 2019 Biofouling. 2019; 55:1-15.

20. Alwaeli Haider A. Al-Khateeb, Susan N. Al-Sadi, Amani. Long-Term Clinical Effect of Adjunctive Antimicrobial Photodynamic Therapy in Periodontal Treatment: A Randomized Clinical Trial. Lasers in Medical Science, 2013. 30(2), 801–807.
21. Qadri T, Miranda L, Tunér J, Gustafsson A. The short-term effects of low-level lasers as adjunct therapy in the treatment of periodontal inflammation. J Clin Periodontol 2005; 32(7):714-719.
22. Cadore UB, Reis MBL, Martins SHL, and et al. Multiple sessions of antimicrobial photodynamic therapy associated with surgical periodontal treatment in patients with chronic periodontitis. J Periodontol. 2019; 90:339–349.
23. Martins, Sérgio H. L.; Novaes, Arthur B.; Taba, Mario; Palioto, Daniela B.; Messora, Michel R.; Reino, Danilo M.; Souza, Sérgio L. S. Effect of Surgical Periodontal Treatment Associated to Antimicrobial Photodynamic Therapy on Chronic Periodontitis: A Randomized Controlled Clinical Trial. Journal of Clinical Periodontology.2017; 40(3):242–251.