Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/14965
DOI URL: http://dx.doi.org/10.21474/IJAR01/14965

RESEARCH ARTICLE

COMPARATIVE EVALUATION OF CLINICAL AND MICROBIOLOGICAL PARAMETERS USING DIODE LASER (970 NM) AS AN ADJUNCT TO SCALING AND ROOT PLANING – A RANDOMIZED CONTROLLED CLINICAL TRIAL

Varsha V., Savita A.M, Pallavi K.N, Sunil S., Archana R.N, Remya R.M, Harsha M.B and Shivaprasad B.M

Manuscript Info

Manuscript History
Received: 25 April 2022
Final Accepted: 27 May 2022
Published: June 2022

Key words:-
Chronic Periodontitis, Scaling and Root Planning, Diode Laser, Polymerase Chain Reaction, Treponema Denticola, Treponema Socranskii

Abstract

Background: Chronic periodontitis (CP) is an infectious disease resulting in inflammation of the supporting tissues of the teeth with progressive attachment loss and bone loss. This study aimed to evaluate the effect of 970-nm diode laser, as an adjunct to scaling and root planing (SRP) in the management of CP in terms of bacterial reduction and changes in clinical parameters.

Methodology: A total of 20 systemically healthy subjects diagnosed with CP were randomly assigned into two groups Group1 (SRP) and Group 2 (SRP and diode laser (970 nm)) with equal numbers in each group. The levels of *Treponema denticola* and *Treponema socranskii* were estimated from plaque samples using real-time polymerase chain reaction. Clinical and microbiological parameters were assessed at baseline and 6 weeks post treatment in both the groups.

Results: Gradual reductions in the levels of *T.denticola*, *T.socranskii*and improvement in the clinical parameters were observed from baseline to 6 weeks in both the groups. However, the comparison between the groups, although clinically relevant, was not found to be statistically significant.

Conclusion: Although no added benefits were found using diode laser (970 nm) application as compared with SRP, it could emerge as an effective non-surgical treatment option in advanced periodontitis with complex inaccessible subgingival niches where comprehensive periodontal care may not be feasible.

Introduction:-

Periodontitis is a polymicrobial infection which is clinically manifested as aninflammatory condition of the toothsupporting structures that leads to a progressive destruction of periodontal tissues and theneventuallytoothloss. The microbialflora in chronicperiodontitis patients consists of a variety of oral bacterial and non-bacterialspecies. *Aggregatibacteractinomycetemcomitans*, *Porphyromonasgingivalis*, spirochaetes, etc., are among the predominantpathogensassociatedwith the severity of periodontal destruction.[1]Oral treponemes, alsoknown as spirochetes, are gram negative, anaerobic, motile, helicalrodsthat are closelyassociatedwithvarious types of periodontaldiseasessuch as gingivitis, necrotizingulcerativegingivitis, and periodontitis.[1]Of thesetreponemes, *Treponemadenticola* and *Treponemasocranskii* are tissue invasive and are frequentlydetected in patients withchronic periodontitis.[2]*Treponema denticola*, a member of the redcomplex group of microorganisms, is a gram negative, obligate, motile, and highlyproteolyticspirochetebacterium which possessnumerous virulence factorslike leucine-

Corresponding Author:- Dr.Varsha Venugopalan

richproteins, outer membrane proteins, dentilisins, trypsin-likeproteaseactivityenhancingits tissue invasive propertythat destroys the periodontal connective tissues.[3]Since, the clinical data regarding the treponemaspecies, especially*T.socranskii*, and itspresence in periodontitis is sparse in the literature, an effort has been made to evaluate the prevalence of *T.socranskii*pre and post treatment. The completeremoval of bacteria and theirtoxinsfromperiodontalpocketsare not alwaysachievedwithconventionalmechanical treatment.[4]Lasers whenused as an adjunct to mechanicaltherapy for periodontaldiseasemayimprove tissue healing by bactericidal and detoxificationeffects. Lasers have the ability to ablate or to vaporizeonly the diseased tissue from the innerepitheliallining of a periodontalpocket by reducing the count of tissue invadersresulting in a better and more predictableresult to the treatment.[4]To the best of ourknowledge, studiesrelated to application of diode laser in reduction of *T.socranskii*has not been reported in the literature. Hence, the primary objective of this split-mouth, randomized, controlledclinical trial was to evaluate the efficacy of diode laser(DL) (970 nm) as an adjunct to SRP in CP patients in terms of bacterialreduction and changes in clinicalparameters. The secondary objective was to compare and correlate the effect of SRP and DL as an adjunct to SRP on clinical and microbiologicalparameters.

Materials and Methods:-

The study design was a split-mouth, randomized, single-centre, controlled clinical trial. The study was conducted on patients who reported to Department of Periodontics, DayanandaSagar College of Dental Sciences, Bangalore, from September 2018 to June 2019. A total number of 56 subjects were screened for eligibility, of which 27 patients met the inclusion criteria and were included in the study. However, only 20 subjects completed the study with a dropout of 7 subjects. (Figure 1) A total of 20 systemically healthy subjects diagnosed with generalized moderate CP in the age group of 30–60 years with a probing pocket depth of ≥5 mm and clinical attachment loss of 3-4 mm were recruited for the study. Patients who had undergone periodontal therapy 6 months prior to the commencement of the study, subjects on antibiotics or immunosuppressants,chronic smokers, alcoholics, smokeless tobacco users, pregnant women and drug abusers were excluded from the study. Ethical clearance was obtained from the ethical committee, DayanandaSagar College of Dental Sciences, Bangalore. Patients were explained about the procedure to be performed and a written informed consent was obtained. Detailed case histories of all patients were recorded which included demographic and clinical parameters.

a) Sample size estimation: -

The sample size was calculated based on data from previous literature study[4] using the formula $N = Z^2 1\text{-}\alpha/2 * P (1\text{-}P) /D2$. Assuming 5% level of significance, 90% power & 10%, sample size calculated was 20.Considering anticipated dropout of 30%, an inflated sample size of 27 was calculated.

b) Method of collection of data:-

This split-mouth study included a total of 20subjects with 120 sites (3 sites per quadrant for each patient) selected based on the inclusion and exclusion criteria which were randomlyassigned with equal numbers to two groups; Group 1(SRP) and Group 2(SRP + DL).The DL used in the study was K-laser with a wavelength of 970 nm and a fiberoptic diameter of 320µm.The laser irradiation was in a contact and continuous mode with a power of 0.9W for 30 seconds on the selected sites in a corono-apical direction. Each site received three applications of 10 seconds each with a time lag of 60 seconds. Clinical parameters such as plaque index (PI), [6] gingival index (GI), [7] probing pocket depth (PPD), and clinical attachment level (CAL) were assessed at baseline and 6 weeks after intervention.

c) Microbial sampling

A total of 80 samples were collected and microbiologically analysed for *T.denticola* and *T.socranskii* levels. Subgingival plaque samples were obtained for microbiological assessment at baseline and 6 weeks after intervention. After adequate isolation, pooled samples were collected from the deepest pockets of each quadrant using area specific curettes and placed in eppendorf tubes containing transport medium TE (tris EDTA) buffer. These samples were stored at -80˚Cuntil they were subjected for real-time polymerase chain reaction (RT-PCR).Patients of both the groups were put on a meticulous oral hygiene regimen and periodically monitored throughout the study period.

d) DNA extraction

DNA extraction from the plaque samples was done using highly purified Invitrogen DNA isolation kit (PureLink™).The standard proteinase K method was followed for DNA isolation. The water bath was set at 55°C. Proteinase K20 µl was added to a sterile microcentrifuge tube. 200 µl of sample was transferred to the tube

containing proteinase K and incubated at 55°C for 30 min. RNAse A 20 µl was added to the lysate and mixed well by briefly vortexing and incubated at room temperature for 2 min. PureLinkGenomicLysis/Binding Buffer 200µlwas added and mixed well by vortexing to obtain a homogenous solution. Ethanol 96%–100% 200 µl was mixed well by vortexing for 5s to obtain a homogeneous solution to proceed for the purification protocol immediately.

e) Purification method

The purification procedure was designed for purifying genomic DNA using a spin column-based centrifugation procedure for a duration of 10-15 min. PureLink™ Spin Column was removed in a collection tube from the package. The entire lysate prepared with PureLink™

Genomic Lysis/Binding Buffer and ethanol was added to the spin column. The column was centrifuged at 10,000 rpmfor 1min at room temperature. The collection tube was placed into a clean PureLink™ collection tube supplied with the kit. An amount of 500 µl of wash bufferprepared with ethanol was added to the column. The column was centrifuged at maximum speed for 3 minutes at room temperature. The collection tube was discarded. The spin column was placed in a sterile 1.5-ml microcentrifuge tube. PureLink™ 25-200 µl genomic elution buffer was added to the column and incubated at room temperature for 1 min. The column was then centrifuged at the maximum speed for 1 min at room temperature, thus obtaining purified genomic DNA in the tube, which was stored at -20 °C until further processing.

f) DNA quantification

Custom SYBR Green assay reagents for *T.denticola* and *T.socranskii*were used in this study. The primer sequences for the two organisms were as follows.

	FORWARD PRIMER	REVERSE PRIMER
T.denticola	3 ' TAATACCGAATGTGCTCATTTACAT5'	5 ‘CTGCCATATCTCTATGTCATTGCTCTT3'
T.socranskii	3 '-AGG TAG ACA GCG GGA AAG GA-5'	5 '-TAA CCC AAC ACC TCA CGG CA-3'

A reaction solution was composed of SYBR Green Universal PCR Master Mix (10 µl), forward primer (1 µl), and reverse primer (1 µl) for *T.denticola* and *T.socranskii*, extracted DNA of unknown sample (1 µl), and nucleus-free water to make a complete reaction volume of 20 µl. The conditions for RT-PCR were as follows: Holding stage at 95°C for 10s followed by 40 cycles of shuttle heating at 95°C for 15s and at 60°C for 1 min. The melt curve stage was at 95°C for 15s, 60°C for 1 min, and 95 °C for 15s. Relative quantity for *T.denticola* and *T.socranskii*was based on the cycle threshold (the number of PCR cycles necessary to obtain the threshold signal of fluorescence) values. All the calculations were done using Applied Biosystems Software.

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 10.5) software. The normality of data was assessed using Shapiro-Wilk test. Intergroup comparisons of clinical and microbiological parameters were done using Student’s unpaired‘t’ test whereas inter-group comparison was done using Student’s unpaired‘t’ test. The possible correlation between clinical parameters (PI, GI, PPD, CAL) and microbiological levels (*T.denticola* and *T.socranskii*) were tested by the Pearson’s correlation test.

Results:-

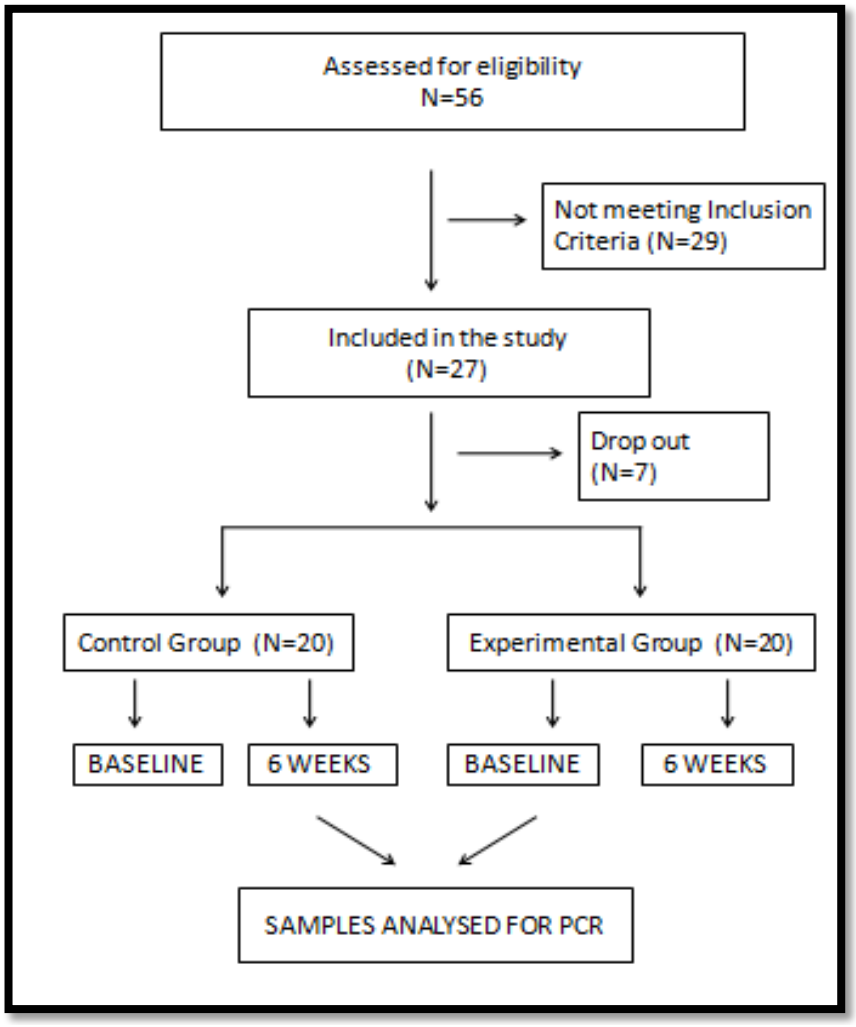

Figure 1:- Participation flow diagram according to the CONSORT 2010 guidelines.

On intragroup comparison, the clinical parameters such as PI, GI, PPD, CAL showed statistically significant results ($p<0.05$) whereas intergroup comparison were statistically insignificant. (Figure 2, 3).

The mean level scores for *T.denticola*in Group 1 at baseline and 6 weeks were 6.04 and 2.435 and for Group 2, they were 5.76 and 1.675 respectively. The intragroup comparison of *T.denticola*levels analysed were statistically significant (P=0.000, P=0.000) and on intergroup comparison, the levels were statistically insignificant (P = 0.88, P=0.29).The mean level scores for *T.socranskii*in Group 1 at baseline and 6 weeks were 5.807 and 2.059 and for Group 2, they were 4.79 and 1.125. The *T.socranskii*levels analysed on intragroup comparison was statistically significant (P=0.000, P=0.000) and intergroup comparison was statistically not significant (P = 0.53, P=0.07). (Figure 4, 5).

The correlation between clinical and microbiological parameters among the two groups was done by using Pearson's correlation test. (TableI). In Group 1, a positive correlation was found between *T.socranskii*and CAL. In Group 2, a positive correlation was found between *T.denticola*and PPD, CAL, GI, PI. Also, *T.socranskii*and CAL values showed positive correlation, both of which were statistically significant.

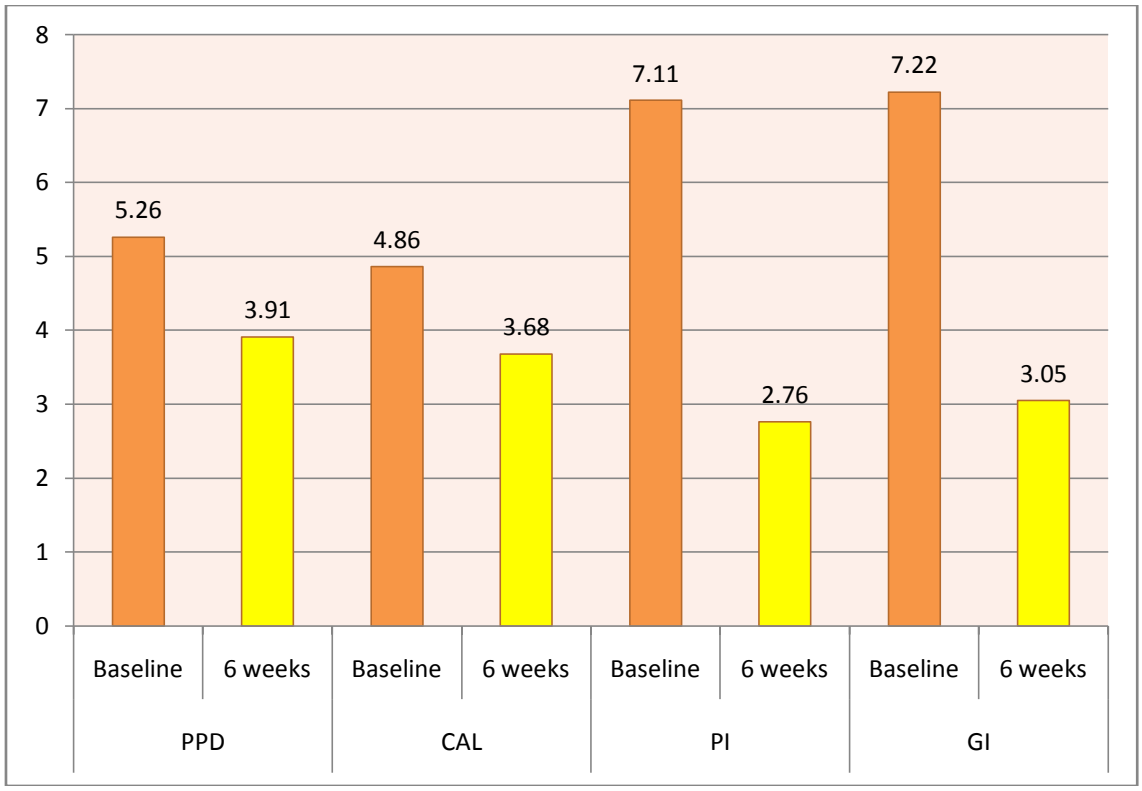

Figure 2:- Comparison of clinical parameters in Group 1.

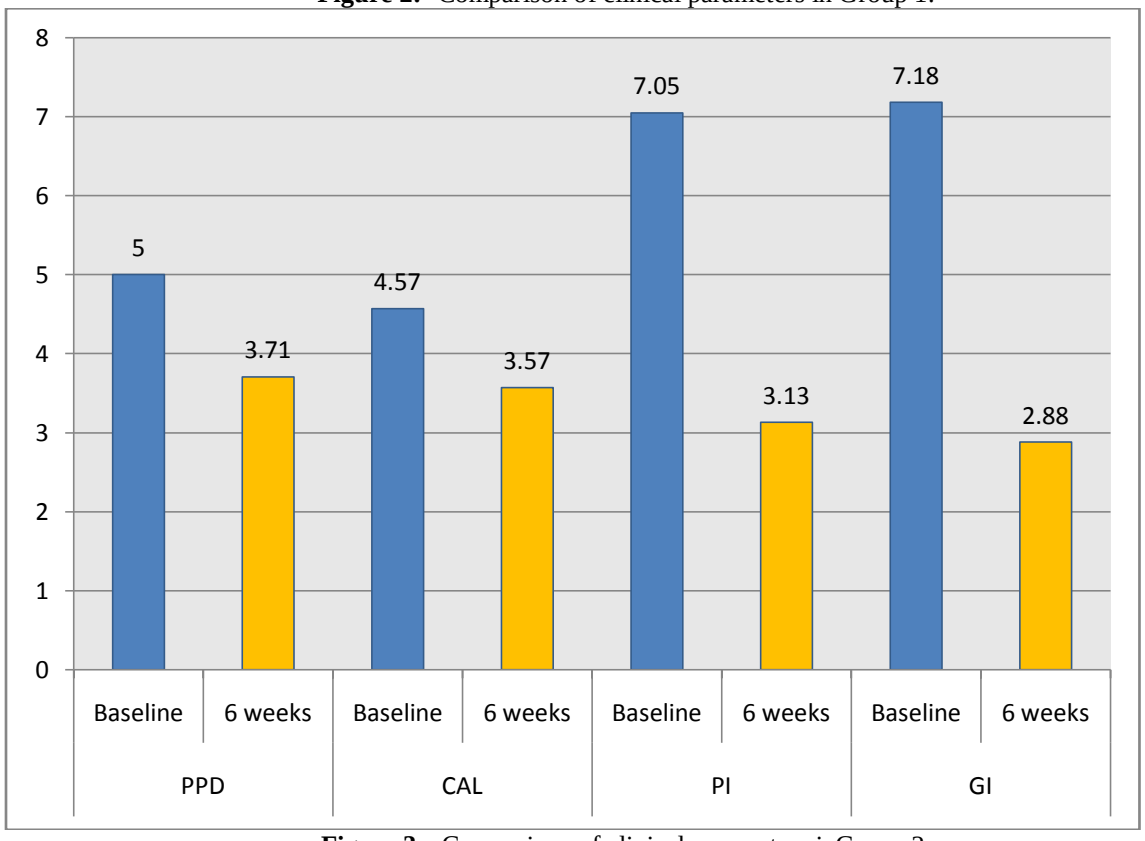

Figure 3:- Comparison of clinical parameters inGroup 2.

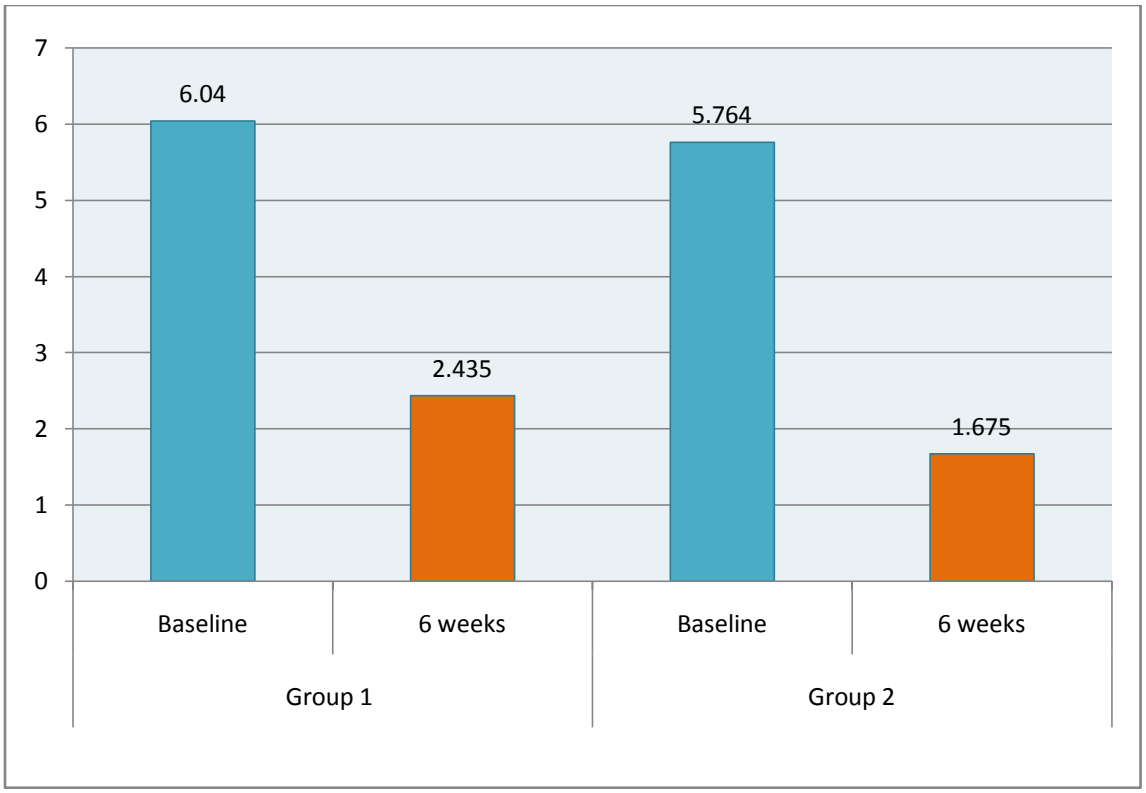

Figure 4:- Comparison of *T.denticola* between groups.

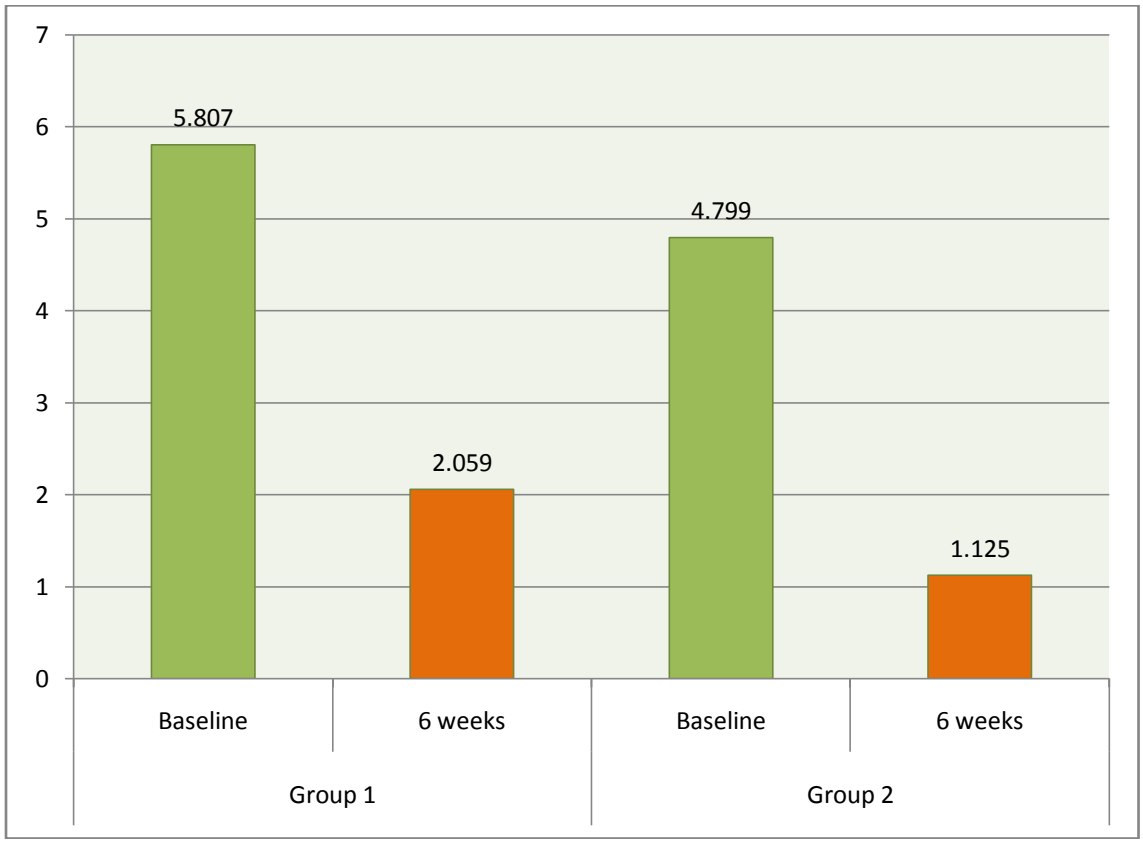

Figure 5:- Comparison of *T.socranskii* between groups.

Table I:- Pearson's correlation between clinical parameters and microbiological analysis in Group 1 and Group 2

GROUP 1								
	Baseline		**6 weeks**		**Baseline**		**6 weeks**	
	T. denticola				***T.socranskii***			
	r value	**p value**	**r value**	**p value**	**r value**	**p value**	**r value**	**p value**
PPD	0.002	0.99	0.06	0.79	0.20	0.93	0.31	0.63
CAL	0.21	0.35	0.17	0.06	0.21	0.37	0.17	0.45
PI	0.11	0.61	0.06	0.79	0.07	0.74	0.03	0.87
GI	0.40	0.07	0.02	0.90	0.39	0.08	0.07	0.97
GROUP 2								
	Baseline		**6 weeks**		**Baseline**		**6 weeks**	
	T. denticola				***T.socranskii***			
	r value	**p value**	**r value**	**p value**	**r value**	**p value**	**r value**	**p value**
PPD	0.20	0.38	0.15	0.00*	0.19	0.41	0.16	0.48
CAL	0.052	0.82	0.48	0.03*	0.12	0.61	0.50	0.04*
PI	0.16	0.49	0.62	0.003*	0.10	0.65	0.38	0.09
GI	0.08	0.71	0.43	0.05*	0.03	0.89	0.17	0.47

Discussion:-

Periodontitis is an infectious disease resulting in inflammation of the supporting tissues of the teeth with progressive attachment and bone loss associated with a complex subgingival ecosystem, predominantly the gram-negative bacterial species. Among these, oral treponemes have been discovered and isolated from the subgingival plaque from diseased periodontal sites.*T. denticola*, an established red complex bacterium is the best characterized and most highly studied species among spirochetes, considered as a putative periodontal pathogen.[8]Also, *T.socranskii*, a gram negative, anaerobic, motile, helical rod, has been shown to be closely associated with periodontal destruction along with other bacteria.[9]Takeuchi et al. identified *T.socranskii* in addition to *T.denticola* and *P.gingivalis* by PCR analysis to be associated with the severity of periodontal destruction along with an exponential increase in clinical parameter values.[10]In a study by Asai et.al, ten subspecies of oral treponemes were detected and quantified from chronic periodontitis patients among Japanese population.[2] Conventional mechanical debridement can achieve a transient decrease in the subgingival level of periodontal pathogens; however, it alone may fail to eliminate tissue invasive microbes as well as pathogenic bacterial niches in the inaccessible areas, such as deep pockets, root concavities, and furcation areas. The use of lasers as an adjunctive therapy for periodontal disease may improve tissue healing by bactericidal and detoxification effects. Lasers have the ability to ablate or vaporize only the diseased tissue from the inner epithelial lining of a periodontal pocket by reducing the count of tissue invaders resulting in a better and more predictable outcome of treatment.[11]

Owing to the importance of knowledge on microorganisms that make up the subgingival microbiota and the limited number of studies involving Indian population, an effort was made to evaluate the prevalence of *T.socranskii*pre and post nonsurgical therapy. In our present study, there was reduction in the clinical parameters (PI, GI, PPD, CAL) at 6 weeks post SRP in both the groupsand were statistically significant in the intragroup comparison & insignificant in the intergroup comparison. Similar findings were observed by a study done by Moritz et.al. and Kamma et.al where the clinical parameters (PI, GI, PPD, CAL) were significantly reduced in the intragroup comparison at 2 weeks and 6 months respectively and insignificant in the intergroup comparison.[12, 13] In contrast to our findings, a study by Carusso et al. showed that the additional treatment with diode laser may lead to a slight improvement of clinical parameters but were statistically insignificant at baseline and 6 weeks.[14]A study done by Alves et.al showed no additional benefits of using diode laser as an adjunct to the conventional periodontal treatment in reduction of PI, GI, PPD, CAL between control group (SRP) and test group (SRP + DL). [15]

In our study, the microbiological analysis presented a statistically significant reduction in the levels of *T.denticola* and *T.socranskii*in the intragroup comparison and was insignificant in the intergroup comparison. Similar studies by

Moritz et.al. wherein different pathogens such as *Aggregatibacteractinomycetemcomitans* and *Porphyromonasgingivalis* levels were studied and a statistically significant reduction in the numbers was observed for both the organisms.[13]In contrast to our findings, studies done on different periodontal pathogens (*Pg,Pi,Aa*) showed no additional benefits of using diode laser as an adjunct to the conventional periodontal treatment in reduction of microbiological levels (after 6 months).[15] Similarly, Carusso et al. showed that the additional treatment with diode laser showed no significant difference between test and control groups in reduction of *Prevotellaintermedia* (*Pi*), *Tannerella forsythia*,*Treponema denticola*, *Fusobacteriumnucleatum*, *Porphyromonasgingivalis*, and *Aggregatibacteractinomycetemcomitans* were found.[14]In the present study, *T.socranskii*was detected in CP patients and a statistically significant reduction was found post treatment in both the groups. We could not compare our results pertaining to *T.socranskii* with other studies since no evidence has been established regarding the levels of *T. socranskii* post diode laser application in the literature and perhaps this study could probably be the first of its kind. A positive correlation was found between the levels of *T. denticola* and GI, PI, CAL suggesting its role in the pathogenesis of periodontal diseases, which was reduced post treatment in both the groups. A similar study done by You et al. showed a positive correlation between *T.denticola* and clinical parameters (PI, GI,PPD, CAL) post SRP.[8]Apart from the diode laser used in our study, other lasers with different wavelengths as an adjunctive therapy for SRP are also reported in the literature. A study by Radavar et al. used Nd:YAG (1068 nm) as an adjunct to SRP and revealed bacterial reduction in the periodontal pockets but was not statistically significant.[17]Another study by Morlock et al.compared diode laser(980 nm) and Nd:YAG(1078 nm) and observed melted and resolidified porous globules consisting of root mineral substance on the root surface after Nd:YAG laser treatment providing an ideal ground for bacterial recolonization, which was not found with diode laser application due toshort wavelength and less heat production.[18]Although Nd:YAG laser is similar to diode laser in its bactericidal effect, it involves temperature increase in deeper tissue layers; whereas in diode lasers, radiation is absorbed by superficial layers, thereby having a better effect on sites affected by periodontal disease.[18]A randomized clinical trial was conducted by Schwarz et.al. using Er:YAG laser as an alternative to SRP where the clinical (PI,GI,PPD,CAL) and microbiological (Colony Forming Units of *Pg*) parameters were significantly reduced.[19]A study by Gutknecht et.al used Er,Cr:YSGG as an adjunct to SRP where he demonstrated a statistically significant reduction in the levels of *Prevotellaintermedia, Tannerella forsythia, Treponema denticola, Fusobacteriumnucleatum, Porphyromonasgingivalis,* and*Aggregatibacter actinomycetemcomitans*.[4]

Although the findings of this study are inconclusive owing to certain limitations like short-term follow up and a smaller sample size, there has been a positive outlook with a definite reduction in the bacterial load and clinical parameters observed at the end of 6 weeks post treatment. Hence, future long-term clinical trials with a larger sample size and treatment using different types of lasers to permit comparisons between studies are needed in order to obtain conclusive results which will guide the treatment of periodontal diseases in the clinical practice.

Conclusion:-

In the present study, nonsurgical periodontal therapy has resulted in an overall improvement in clinical parameters as well as reduction in the microbial load of *T.denticola* and *T.socranskii* at the end of 6 weeks. The DL application used as an adjunct to SRP has led to a clinically significant improvement in both clinical and microbial parameters as compared with SRP alone which were statistically significant with intragroup comparison. Although lasers have not been found to be effective in the present study, it has the potential to emerge as an effective non-surgical treatment modality in patients with multiple, complex, inaccessible subgingival niches who prefer non-surgical treatment as well as in medically compromised patients in whom standard comprehensive periodontal care may not be feasible, thereby reducing the risk of bacteraemia. However, long-term prospective trials with large sample size are needed to affirm that the diode laser can be used as an adjunct to SRP in the treatment of periodontal diseases.

Funding sources:
No financial support was received for this study.

Conflict of interest:
None.

Registered Clinical Trial no: -
CTRI/2018/08/015407

References:-

1. Abdul Jabbar T et.al. Role of lasers as an adjunct to scaling and root planing in patients with type 2 diabetes mellitus: a systematic review. Lasers Med Sci 2016.
2. Asai Y, Jinno T, Igarashi H, Ohyama Y, Ogawa T. Detection and Quantification of Oral Treponemes in Subgingival Plaque by Real-Time PCR. J.Clin.Microbiol 2002; 40:3334-40.
3. Scott D, Siboo IR, Chan ECS, Siboo R. An extracellular enzyme with hyaluronidase and chondroitinase activities from some oral anaerobic spirochaetes. Microbiology 1996; 42:2567-76. Gutknecht N, Betteray CV, Ozturan S, Vanweersch L, FranzenR.Laser Supported Reduction of Specific Microorganisms in the Periodontal Pocket with the Aid of an Er,Cr:YSGG Laser: A Pilot Study. The Scientific World Journal; 2014:1-7.
4. Armitage GC. Periodontal diagnosis and classification of periodontal diseases.Periodontal 2000.2004; 34:9-21.
5. Silness J, Loe H. Periodontal disease in pregnancy.Correlation between oral hygiene and periodontal condition. ActaOdontol 1964; 22:121-135.
6. Loe H, Silness J. Periodontal disease in pregnancy.Prevalence and severity.ActaOdontolScand 1963; 21:533-51.
7. You M, Leung KW, Watt RM. Comparative analysis of oral treponemes associated with periodontal health and disease. BMC Infectious Diseases.2013; 13:174-9.
8. Smibert M R, Johnson J L, Ranney RR. Treponema socranskii sp. nov. Treponema socranskii subsp.socranskii subsp. nov. Treponema socranskii subsp. buccalesubsp.nov. and Treponema socranskii subsp. paredis subsp. nov. Isolated from the Human Periodontia. INTERNATIONAL JOURNAL OF SYSTEMIC BACTERIOLOGY.1984; 34:457-462.
9. Takeuchi Y, Umedha M, Sakamoto M, Benno Y, Huang Y, Ishikawa I. Treponema socranskii, Treponema denticola and Porphyromonasgingivalis are associated with severity of periodontal destruction. J Periodontal.2001; 10:1354-63.
10. Ishikawa I, Aoki A, Takasaki AA, Mizutani K, Sasaki KM, Izumi Y. Application of lasers in periodontics: True innovation or myth? Periodontol 2000.2009; 50:90-126.
11. Kamma JJ, Vasdekis V, Romanos GE. The Effect of Diode Laser (980 nm) Treatment on Aggressive Periodontitis: Evaluation of Microbial and Clinical Parameters. Photomedicine and Laser Surgery.2009; 27:11-19.
12. Moritz A, Schoop U, Goharkhay K, Schauer P. Treatment of periodontal pockets with a diode laser. Lasers in Surgery and Medicine.1998; 22:302-11.
13. Caruso U, Nastri L, Piccolomini R, d'Ercole S, Mazza C, Guida L. Use of diode laser 980 nm as adjunctive therapy in the treatment of chronic periodontitis. NEW MICROBIOLOGICA 2008; 31:513-18.
14. Alves V et.al. Clinical and microbiological evaluation of high intensity diode laser adjutant to non-surgical periodontal treatment: a 6-month clinical trial. Clin Oral Invest.2013; 17:87-95.
15. Borrajo JL, Garciavarela L, Castro LG, Rodrigueznunez, Torreira GM. Diode Laser (980 nm) as Adjunct to Scaling and Root Planing .Photomedicine and Laser Surgery. 2004; 22:509-12.
16. M Radvar et.al. An evaluation of the Nd: YAG laser in periodontal pocket therapy. Br Dent J.1996; 180:57-62.
17. Morlock J, Pippin DJ, Cobb CM, Killoy WJ, Rapley JW. The effect of Nd: YAG Laser exposure on root Surfaces when used as an Adjunct to Root Planing: An in vitro study. J Periodontol.1992; 63:637- 41.
18. Schwarz F, Sculean A, George T, Reich E. Periodontal treatment with Er: YAG Laser compared to Scaling and Root planing .A Controlled Clinical Study. J Periodontol.2001; 72:361-67.