ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 3, Issue – 1, January - 2020, Page No. : 77 - 84

Photodynamic therapy and its effect on oxidative stress following nonsurgical therapy in patients with chronic periodontitis: A Clinico-Biochemical study

[1]Dr Pooja Palwankar, Prof and Head, Department of Periodontology, Manav Rachna Dental College, Faridabad, Haryana, India

[2]Dr Karunik Gupta, Postgraduate student Department of Periodontolgy, Manav Rachna Dental College, Faridabad, Haryana, India

[3]Dr Anjana Goyal, Reader Department of Biochemistry, Manav Rachna Dental College, Faridabad, Haryana, India

[4]Dr Lipika Gopal, Senior lecturer Department of Periodontology, Manav Rachna Dental College, Faridabad, Haryana, India

Corresponding Author: Dr Pooja Palwankar, Professor and Head, Department of Periodontology, Manav Rachna Dental College. Faridabad, Haryana, INDIA

Citation of this Article: Dr Pooja Palwankar, Dr Karunik Gupta , Dr Anjana Goyal, Dr Lipika Gopal, "Photodynamic therapy and its effect on oxidative stress following nonsurgical therapy in patients with chronic periodontitis: A Clinico-Biochemical study", IJDSIR- January - 2020, Vol. – 3, Issue -1, P. No. 77 – 84.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Aim: To evaluate the efficacy of photodynamic therapy and its effect on oxidative stress in gingival crevicular fluid following nonsurgical therapy in patients with chronic periodontitis.

Methods and Material: 108 sites in 18 chronic periodontitis subjects were assigned to three groups. Test group 1 (PDT with placebo + SRP), Test group 2 (PDT with indocyanine green dye + SRP), and control group (SRP only). The clinical parameters GI, PI, CAL, PD, reduced and oxidised glutathione and redox balance in GCF were recorded at baseline, 1st month and 3rd month.

Statistical analysis: One way Anova, Kruskal Wallis test and Mann Whitney u test for intergroup & Wilcoxson signed ranks test for intragroup comparison. A statistical significance p <0.01were considered significant at 99% confidence interval.

Results: Test group 1 showed significant improvement in the GI, PI, CAL, PD and oxidative stress levels and improved redox balance at 1 and 3 months in comparison with the other two groups.

Conclusions: Photodynamic therapy with Indocyanine green dye in adjunct to SRP was more effective than photodynamic therapy with SRP and SRP alone.

Key-words: GCF- Gingival crevicular fluid, ROS- Reactive oxygen species, PDT– Photodynamic therapy, ICG– Indocyanine green dye, SRP- Scaling and root planing, PI- plaque index, GI- gingival index, PD- Pocket

depth, CAL- clinical attachment level, OS – Oxidative stress.

Introduction

Periodontitis is a chronic inflammatory disease that affects the supporting structures of the teeth, resulting in tooth loss. Tissue destruction in periodontitis appears to be largely mediated by an abnormal host response to specific bacteria and their products. The primary etiologic agents are predominantly Gram negative anaerobic or facultative anaerobic bacteria within the biofilm.1,2 Inflammatory cells form a major portion of the host immune response and neutrophils being the predominant inflammatory cells (Kawashi, 1980) act via 3 different mechanisms:- Synthesis of reactive oxygen species, Secretion of lysosomal enzymes, Oxygen dependent lipid peroxidation (Cyril, 1995).[3]

The role of the antioxidant in defense system is to protect vital cell and tissue structures and bio-molecules from host- derived reactive oxygen species (ROS). Generation of reactive oxygen species is thought to be a major factor in the aetiology of local tissue damage. Glutathione levels in GCF were first detected by Chapple in 1996. Glutathione present in gingival crevicular fluid is reported to be one of the most important redox regulators which control the inflammatory process.[4] It maintains antioxidant stress levels.

The debridement in the form of scaling and root planing (SRP) is considered as a gold standard but alone may not be sufficient to kill the pathogens.[5] Laser therapy, is advocated as an adjunct to conventional therapy and has been proposed as an innovative treatment option in controlling the subgingival microorganisms. The effect of lasers are enhanced by combining them with photosensitizing agents, which can result in photo destruction of target cells due to their cytotoxic effects in the presence of oxygen. This application of light over photosensitizing dye is Photodynamic therapy (PDT). PDT involves the combination of visible light, usually with a diode laser and a photosensitizer.[6] (Fig-1)

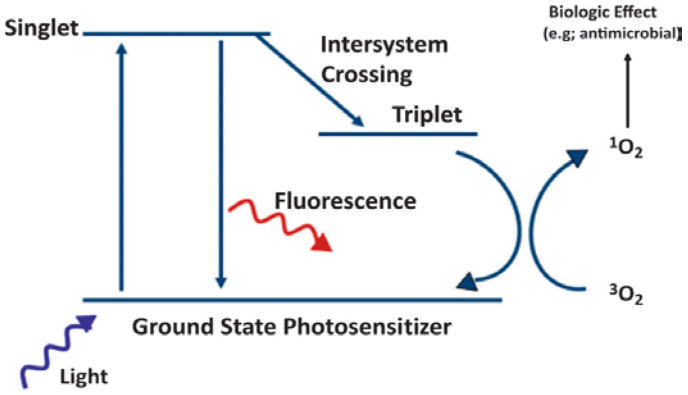

Fig.1: The working principal of LASER upon photosensitizing agent and activating it to the triplet state thus leads to the killing of anaerobic subgingival pathogens

A new photosensitiser called Indocyanine green (ICG), a tri-carbocyanine that belongs to the family of cyanine dyes has been developed.[7] It is widely used for determining cardiac output, hepatic function and liver blood flow, and for ophthalmic angiography. Recent in vitro studies have reported its efficacy in killing potent periodontal pathogens.

The present study was undertaken to evaluate the efficacy of photodynamic therapy using indocyanine green dye and its effect on oxidative stress in the form of glutathione in gingival crevicular fluid following nonsurgical therapy in patients with chronic periodontitis.

Material and Methods

A clinical study was undertaken for 18(108 sites) chronic periodontitis patients of mean age 40 years with probing pocket depth of 5-7mm after approval from institutional ethical committee and number was MRDC/2016/IEC/09. Six sites from each of the patients were computer randomised to receive SRP only (control), SRP + PDT (940nm Laser) with placebo dye (Test Group -1) and SRP

+ PDT (940nm Laser) with ICG Aurogreen®(Test Group -2). Patients who gave informed consent were enrolled for the study. Patients who have undertaken periodontal therapy in last 6 months and history of allergy to antioxidant/ iodides, systemic diseases, tobacco users and lactating mothers were excluded from the study.(Fig-2)

18 Subjects (108 sites)

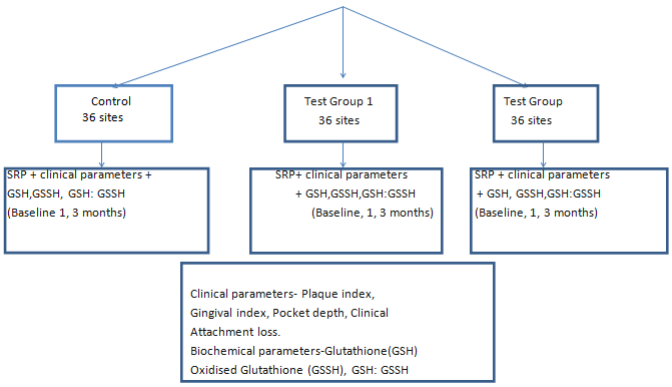

Fig. 2: Flow chart

Preparation of Indocyanine Green (ICG) solution and Placebo solution - ICG powder was dissolved in 50 ml of distilled water to prepare an initial 25mg/ml ICG stock solution. The stock was then diluted in balanced saline solution to achieve the final ICG concentrate of 5mg/ml. Placebo was prepared by using the artificial food colouring agent of green colour. 1gm of powder was dissolved in 10ml of distilled water. Since aqueous solution of ICG is unstable and have to be used within 6 hours, fresh solutions have to be made at every application.

Dispensing of dyes

The ICG & Placebo were dispensed in sterile syringes and labelled as solution A and B respectively.

Method

At baseline, all patients received a full mouth scaling and root planing. All the clinical parameters and levels of antioxidant levels were estimated at the baseline 1 & 3 months. The probing depth was standardized using a customized acrylic stent; the lower margin of the stent was the reference point and groove as reference for angulation of probe insertion. The probing pocket depth (PD) was measured as the distance from the marginal gingiva to the base of the pocket.

At baseline, Indocyanine green dye or placebo were introduced into the deepest area of the periodontal pocket with a blunted needle until the dye reached the superior portion of the gingival crevice at the sites after this the Low Level Laser (Diode Laser) beam at 940 nm in a continuous contact mode with 0.7 W output for 5 seconds was applied. Decoding was done to compare and relate the data

Biochemical Analysis

Evaluation of glutathione level - Reduced (GSH) and Oxidized Glutathione (GSSG) levels was done using Beutler's method[8] using spectrophotometric test using a chromogenic agent- Elliman`s agent.8 chemical used were Tricitric acid (TCA),Ethylenediaminetetra-acetic acid(EDTA), Tris (EDTA), distilled water, Hydrochloric acid, absolute Ethanol & 5,5 -dithiobis(2- Nitrobenzoic acid)(DTNB).

Sampling for biochemical analysis

1µl GCF collected with the micropipettes from the sites was transferred to Eppendorf tubes after adding 99 µl of distilled water. The sample in Eppendorf tube is inverted in a test tube "T". The Standard test tube "S" was prepared by adding 100 µl of standard solution and the blank test tube B was prepared by adding 90 µl of distilled water. Then, 800 µl of distilled water was added the test and standard test tubes to make the amount of sample equal. 100 µl of TCA was added to all test tubes and then kept in vortex shaker for 10-15 minutes. The test tubes were then centrifuged for 15 minutes at 3000rpm. 400 µl of supernatant was removed from all the three test tubes and 800 µl of Tris - EDTA was added. Finally 20 µl of DTNB

solution was added to all tubes. The tubes were kept out of the vertex shaker and readings were recorded within 2-3 minutes in a spectrophotometer at 412 nm wavelength.

Results

The gingival index (GI) Loe and silness and plaque index (PI) Turesky modification of Quigley and Hein and pocket depth of all the three groups showed a reduction in respective scores from baseline to 3 months. However, there was no statistically significant difference between the 3 groups.

Descriptives		CAL (Baseline)		CAL (1st Month)		CAL (3rd Month)	
	N	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
Control Group	36	6.06	0.83	5.03	0.74	4.62	0.65
Test Group 1	36	6.00	0.83	5.06	0.75	4.65	0.69
Test Group 2	36	6.28	0.91	5.17	0.81	4.71	0.72
Total	108	6.11	0.86	5.08	0.76	4.66	0.68
F		1.059		0.33		0.145	
p-value		0.35 (NS)		0.72 (NS)		0.865 (NS)	
Control Group vs	Test Group 1	0.959 (NS)		0.987 (NS)		0.983 (NS)	
Control Group vs	Test Group 2	0.516 (NS)		0.724 (NS)		0.857 (NS)	
Test Group 1vs	Test Group 2	0.358 (NS)		0.813 (NS)		0.934 (NS)	
(S- Significant), (NS- Not Significant) p<0.01							

Table 1: Intergroup comparison of clinical attachment level (CAL)

A gain in Clinical attachment level (CAL) was seen from baseline to 3 months with the greatest reduction seen in test group 2 followed by the control group and least in test group1 (Table-1). But, there was no statistically significant difference among the groups.

Descriptives		Baseline GSH		GSH 1month		GSH 3month	
	N	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
Control Group	18	125.78	31.36	281.44	135.43	400.53	134.21
Test Group 1	18	155.94	75.60	381.67	92.72	502.65	94.55
Test Group 2	18	160.17	62.71	387.44	96.90	508.76	109.92
Total	54	147.30	60.41	350.19	118.60	470.65	122.49
F		1.786		5.28		4.83	
p-value		0.178 (NS)		0.008 (S)		0.012 (S)	
Control Group vs	Test Group1	0.29 (NS)		0.023 (S)		0.032 (S)	
Control Group vs	Test Group2	0.203 (NS)		0.015 (S)		0.021 (S)	
Test Group 1 vs	Test Group2	0.975 (NS)		0.986 (NS)		0.987 (NS)	
(S- Significant), (NS- Not Significant) p<0.01							

Table 2: Intergroup comparison of Reduced Glutathione (GSH)

The 3 groups showed an increase in reduced glutathione level (GSH) from baseline to 3 months. No statistically significant difference was seen in the GSH values of the 3 groups when compared. At 3 months interval, the GSH level was greater in test group 2 followed by the test group 1 and the control group. There was significant difference seen between control and test group 1& 2, with no significant difference between test group1 & test group 2 (Table-2)

Descriptives		Baseline GSSG		GSSG 1month		GSSG 3month	
	N	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
Control Group	18	335.83	102.75	146.78	81.69	111.06	76.50
Test Group 1	18	299.44	88.73	137.67	65.10	67.12	16.07
Test Group 2	18	332.33	127.06	150.00	61.42	64.71	21.96
Total	54	322.54	106.60	144.81	68.83	80.96	50.72
F		0.629		0.15		5.266	
p-value		0.537 (NS)		0.861 (NS)		0.009 (significant)	
Control Group vs	Test Group 1	0.57 (NS)		0.919 (NS)		0.023 (significant)	
Control Group vs	Test Group 2	0.995 (NS)		0.99 (NS)		0.016 (significant)	
Test Group 1	Test Group 2	0.631 (NS)		0.858 (NS)		0.988 (NS)	
(S- Significant), (NS- Not Significant) p<0.01							

Table 3: Intergroup comparison of Oxidised Glutathione (GSSG)

Decrease in oxidized glutathione level (GSSG) was seen in all the 3 groups from baseline to 3 months. But, no statistical significant difference was found at baseline and 1 month. At 3 months the GSSG values were least in the

test group 2 followed by the test group 1 and control group. The difference was significant when the control group was compared with the both the test groups, with no difference between test groups 1 & 2(Table-3).

Descriptives		Baseline GSH/GSSG		GSH/GSSG 1month		GSH/GSSG 3 month	
GSH: GSSG	N	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
Control Group	18	0.40	0.13	2.36	1.45	4.78	2.88
Test Group 1	18	0.53	0.20	3.28	1.32	7.92	2.36
Test Group 2	18	0.58	0.39	3.00	1.50	8.73	3.03
Total	54	0.50	0.27	2.88	1.45	7.14	3.22
F		2.207		1.979		9.664	
p-value		0.12 (NS)		0.149 (NS)		0.001 (S)	
Control Group vs	Test Group 1	0.306 (NS)		0.138 (NS)		0.005 (S)	
Control Group vs	Test Group 2	0.116 (NS)		0.373 (NS)		0.001 (S)	
Test Group 1 vs	Test Group 2	0.851 (NS)		0.828 (NS)		0.673 (NS)	
(S- Significant), (NS- Not Significant) p<0.01							

Table 4: Intergroup comparison of Ratio of reduced Glutathione and Oxidised Glutathione

All the 3 groups showed change in redox balance GSH: GSSG level from baseline to 3 months, but no statistically significant difference was seen at baseline and 1 month. At 3 months the ratio of GSH :GSSG was greater in test group 2 followed by test group 1 & control group. There was significant difference between control and test group 1 & 2, with no difference between test group 1 & test group 2 individually (Table-4).

Discussion

Photodynamic therapy (PDT) involves the combination of visible light, usually through the use of a diode laser and a photosensitizer.[9] Chan Y, Lai CH (2003) [10] conducted a study to clarify bactericidal effects of photodynamic therapy and to eliminate periodontal pathogens, stated that using a diode laser of proper power and wavelength could be a useful adjunct to mechanical debridement in the prevention of the re-colonisation of subgingival lesions by pathogenic microorganisms. Meisel P, Kocher T (2005) [11] conducted a study to evaluate the efficacy of photodynamic therapy (PDT) as an alternate to the systemic antibacterial drugs used in the treatment of periodontal diseases. They stated that the photodynamic therapy may be used as an adjunct to conventional antibacterial measures in periodontology.

Few of the variables, including clinical attachment level (CAL) and probing depth (PD) measurements and the presence of bleeding on probing (BOP) are commonly used to assess and monitor the periodontal status. Biomarkers like vitamins, coenzyme Q10, urate, reduced glutathione, oxidized glutathione, superoxide dismutase, catalase, glutathione peroxidase etc. have been used to assess the periodontal status. [12] Damage mediated by free radicals can be mitigated by antioxidant defense systems present in the body. Glutathione exists as Oxidized glutathione (GSSG) and Reduced glutathione (GSH) as an important anti-oxidant, and plays a role in the detoxification.[13] Grant MM, Brock GR, Matthews JB, Chapple ILC (2010)[14] in their study to quantify reduced and oxidized glutathione (GSH and GSSG) levels in gingival crevicular fluid (GCF) of periodontitis patients. The Redox balance can be upto 1:12 in healthy human GCF.

The greatest reduction in plaque index scores was seen in test group 2 followed by test group 1 and the least in the control group. These findings were similar to Qadri et al (2003)[15] & P Chondros(2009)[16] who reported a significant decrease in plaque index values on the laser treated side. The greatest reduction in gingival scores was seen in the control group followed by test group 2 and test group 1 with no statistically significant difference between the three groups. These findings were in accordance with those of Theodoro et al (2012)[17], who stated that a single PDT session after SRP treatment did not result in a significant reduction in clinical signs of inflammation in the patients with chronic periodontitis compared with SRP alone. Studies by M Berakdar et al (2012) [18], I Cappuyns

(2012) [19], A Dilsiz (2013) [20] also found no significant difference between the effect of photodynamic therapy / laser on gingival scores as compared to SRP alone.

All the three groups showed significant reduction in probing depth from baseline to 1 and 3 months. The greatest reduction was seen in test group 2 followed by the control group and test group 1. Studies by Chan Y (2003) [21], M Lulic (2009) [22] showed similar results . All three groups showed a gain in Clinical attachment from baseline to 3 months, the greatest gain in Clinical attachment seen in test group 2 followed by test group1 and control group However, there was no statistically significant difference between the groups. This gain in clinical attachment in test group 2 might be because of the sterilizing effect of photodynamic therapy that would have reduced the anaerobic bacteria and lead to the healing in the 3 months duration. Almost similar results were observed in the studies conducted by Braun A (2008) [23] Rühling A (2010) [24]

A significant increase in GSH and decrease in GSSG was observed along with improvement in the GSH: GSSG ratio following photodynamic therapy with indocyanine green dye, adjunct to SRP than photodynamic therapy with placebo and only SRP group. Improvement of redox status (GSH: GSSG) may be due to reduction in amount of GSSG & maintenance of GSH within GCF after treatment. These above results are indicative of the fact that following treatment the pocket epithelium is free from oxidative stress. Tissue irradiation is achieved through the medium of a photosensitizer dye that selectively penetrates into the deeper tissues and specifically binds to the bacterial cell wall. Upon photoexcitation after interaction with a light of a particular wavelength, there is a generation of cytotoxic singlet oxygen and reactive oxygen species which cause pronounced antimicrobial action at the treatment site. As a result of the cytotoxic nature of the singlet oxygen, it is unlikely that the microorganisms would develop resistance to it. Furthermore, host tissue damage is not encountered due to the protective presence of keratin that inhibits the cytotoxic activity, thus promoting selective bacterial killing. This may be one of the possible causes of results observed at 3 months duration. Studies by I Borges Jr. et al (2007)[25], MM Grant et al (2010)[26], PS Palwankar et al (2015)[27, 28] showed similar results.

Conclusion

Within the limitations of this study, it is recommended that the adjunctive use of photodynamic therapy using Indocyanine green dye can boost the antioxidant concentration and it could also be effective in improving the clinical parameters following the treatment of chronic periodontitis patients. Longitudinal studies using a larger sample size are required to ascertain the effect of photodynamic therapy using (Indocyanine green dye) as an adjunct to SRP and on oxidative stress, in the treatment of chronic periodontitis.

References

1. Dahiya P, Kamal R, Gupta R, Bhardwaj R, Chaudhary K, Kaur S. Reactive oxygen species in periodontitis. J Indian Soc Periodontol. 2013 Jul;17(4):411.

2. Battino M, Bullon P, Wilson M, Newman H. Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species. Crit Rev Oral Biol Med.1999 Jul;10(4):458-76.

3. Fujita T. Formation and removal of reactive oxygen species, lipid peroxides and free radicals, and their biological effects. Yakugaku Zasshi. 2002 Mar 1;122(3):203-18.

4. Chapple ILC, Brock G, Eftimiadi C, Matthews JB. Glutathione in gingival crevicular fluid and its relation to

local antioxidant capacity in periodontal health and disease. Mol Pathol. 2002 Dec;55(6):367.

5. Ruiz Magaz V. Efficacy of adjunctive Er, Cr: YSGG laser application following scaling and root planing in periodontally diseased patients (Doctoral dissertation, Universitat Internacional de Catalunya).

6. Christodoulides N, Nikolidakis D, Chondros P, Becker J, Schwarz F, Rössler R, Sculean A. Photodynamic therapy as an adjunct to non-surgical periodontal treatment: a randomized, controlled clinical trial. J Periodontol. 2008 Sep;79(9):1638-44.

7. Valente NA. Comparative evaluation of two different diode laser wavelengths with and without photosensitization for suppressing bacterial colonization on dental implants surfaces. Ex-vivo study (Doctoral dissertation, State University of New York at Buffalo).

8. Beutler E. Improved method for determination of blood glutathione. J Lab Clin Med. 1963;61(5):882-8.

9. Castano AP, Demidova TN, Hamblin MR. Mechanisms in photodynamic therapy: part one-photosensitizers, photochemistry and cellular localization. Photodiagnosis Photodyn Ther. 2004 Dec 1;1(4):279-93.

10.Chan Y, Lai CH. Bactericidal effects of different laser wavelengths on periodontopathic germs in photodynamic therapy. Lasers Med Sci. 2003;18(1):51-5.

11.Meisel P, Kocher T. Photodynamic therapy for periodontal diseases: state of the art. J Photochem Photobiol. 2005;79(2):159-70.

12.Strimbu K, Tavel JA. What are biomarkers? Curr Opin HIV AIDS. 2010;5(6):463-6

13.Durantini AM, Greene LE, Lincoln R, Martinez SR, Cosa G. Reactive oxygen species mediated activation of a dormant singlet oxygen photosensitizer: from autocatalytic singlet oxygen amplification to chemicontrolled photodynamic therapy. J Am Chem Soc. 2016;138(4):1215-25.

14.Grant MM, Brock GR, Matthews JB, Chapple ILC. Crevicular fluid glutathione levels in periodontitis and the effect of non-surgical therapy. J Clin Periodontol 2010;37(1):17-23.

15.Chondros P, Nikolidakis D, Christodoulides N, Rössler R, Gutknecht N, Sculean A. Photodynamic therapy as adjunct to non-surgical periodontal treatment in patients on periodontal maintenance: a randomized controlled clinical trial. Lasers Med Sci. 2009;24(5):681-8.

16.Theodoro LH, Silva SP, Pires JR, Soares GH, Pontes AE, Zuza EP, Spolidório DM, de Toledo BE, Garcia VG. Clinical and microbiological effects of photodynamic therapy associated with nonsurgical periodontal treatment. A 6-month follow-up. Lasers Med Sci. 2012;27(4):687-93.

17.Berakdar M, Callaway A, Eddin MF, Roß A, Willershausen B. Comparison between scaling-root-planing (SRP) and SRP/photodynamic therapy: six-month study. Head Face Med 2012;8(1):12.

18.Cappuyns I, Cionca N, Wick P, Giannopoulou C, Mombelli A. Treatment of residual pockets with photodynamic therapy, diode laser, or deep scaling. A randomized, split-mouth controlled clinical trial. Lasers Med Sci 2012;27(5):979-86.

19.Dilsiz A, Canakci V, Aydin T. Clinical effects of potassium–titanyl–phosphate laser and photodynamic therapy on outcomes of treatment of chronic periodontitis: a randomized controlled clinical trial. J Periodontol 2013;84(3):278-86.

20.Chan Y, Lai CH. Bactericidal effects of different laser wavelengths on periodontopathic germs in photodynamic therapy. Lasers Med Sci. 2003;18(1):51-5.

21.Lulic M, Leiggener Görög I, Salvi GE, Ramseier CA, Mattheos N, Lang NP. One-year outcomes of repeated adjunctive photodynamic therapy during periodontal

maintenance: a proof-of-principle randomized-controlled clinical trial. J Clin Periodontol 2009;36(8):661-66.

22.Braun A, Dehn C, Krause F, Jepsen S. Short-term clinical effects of adjunctive antimicrobial photodynamic therapy in periodontal treatment: a randomized clinical trial. J Clin Periodontol 2008;35:877-84.

23.Rühling A, Fanghänel J, Houshmand M, Kuhr A, Meisel P, Schwahn C, Kocher T. Photodynamic therapy of persistent pockets in maintenance patients—a clinical study. Clin Oral Investig. 2010;14(6):637-44.

24.Borges Jr I, Moreira EA, De Oliveira TB, da Silva MB, Fröde TS. Proinflammatory and oxidative stress markers in patients with periodontal disease. Mediators of Inflammation. 2007;2007:1-5.

25.Grant MM, Brock GR, Matthews JB, Chapple IL. Crevicular fluid glutathione levels in periodontitis and the effect of non-surgical therapy. J Clin Periodontol 2010;37(1):17-23.

26.Palwankar P, Rana M, Arora K, Deepthy C. Evaluation of non-surgical therapy on glutathione levels in chronic periodontitis. J Euro Gen Dent 2015;9(3):415-422.

27.Palwankar P, Gopal L, Verma A. Effect of Green Tea catechin Gel On Crevicular Glutathione Levels In chronic Periodontitis - A Clinico-Biochemical Study. IJSR;2018:7(8):14-18.