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REVIEW ARTICLE **OPEN ACCESS**

Efficacy of Methylene Blue in Antimicrobial photodynamic therapy (aPDT) for management of Periodontitis - A systematic review

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ABSTRACT
Periodontitis is a chronic, multifactorial and polymicrobial inflammatory disease which is characterized by destruction of periodontal ligament, alveolar bone and is associated with gingival inflammation, pocket formation and gingival recession. The elimination of the supragingival and subgingival microbial biofilm is the main focus for treatment of periodontitis. aPDT can be defined as the eradication of target cells by reactive oxygen particles produced by means of a photosensitizing compound and light of an appropriate wavelength. The aim of this review is to determine the efficacy of methylene blue in antimicrobial photodynamic therapy (aPDT) for treatment of periodontitis. The following combinations of Medical Subject Heading Terms (MeSH) and keywords were used (Photochemotherapy) AND (Periodontitis) AND (Methylene Blue) AND (Photosensitizing agents) AND (Periodontal pocket) AND (Photodynamic therapy). Twenty-four articles were included in this systematic review. aPDT was carried out in the included studies using methylene blue as a photosensitizer. aPDT carried out as an adjunct to Scaling and root planning provided better outcomes as compared to Scaling and root planning alone. Methylene blue mediated aPDT can be a treatment modality for the management of periodontitis.
Keywords: *Periodontitis, Methylene Blue, Photosensitizing agents, Periodontal pocket, Photodynamic therapy.*

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INTRODUCTION

Periodontitis is a chronic, multifactorial and polymicrobial inflammatory disease which is characterized by destruction of periodontal ligament, alveolar bone and is associated with gingival inflammation, pocket formation and gingival recession. The elimination of the supragingival and subgingival microbial biofilm is the main focus for treatment of periodontitis. Scaling and root planing (SRP) is the mechanical debridement of tooth and root surfaces allowing sufficient cleaning of the periodontal pockets and facilitating periodontal reattachment [1]. Presence of deep pockets, furcation areas and root curvatures are difficult to access by SRP and cannot completely remove the biofilm. Local drug delivery (LDD) of antimicrobial agents directly into periodontal pockets has been suggested as an alternative to systemic antibiotics [2]. However, LDD can be difficult in application to multiple sites of deep pockets in cases of generalized periodontitis. To overcome these complications related to the local and/or systemic use of antibiotics, Antimicrobial photodynamic therapy (aPDT) was suggested to provide a mean of killing microbes in localized topical infections [3]. aPDT can be defined as the eradication of target cells by reactive oxygen particles produced by means of a photosensitizing compound and light of an appropriate wavelength [4]. The mechanism of aPDT involves the use of a photosensitizer that directly target both Gram-negative and Gram- positive bacteria without affecting the host cells. The photosensitize reacts with oxygen on activation by light and produces a highly reactive state of oxygen known as singlet oxygen, which is toxic to microorganisms. Thus , aPDT reduces microbial load [5]. The commercial phenothiazine dye i.e Methylene Blue (MB) is an effective photosensitizing agent for the inactivation of pathogenic organisms, including viruses, bacteria, and yeast [6]. Methylene blue combined with light has been reported to kill C. albicans too [7]. With laser activation, MB can produce a variety of reactive oxygen species including singlet oxygen molecules, superoxide anion radicals (O2··), and hydroxyl radicals (OH·) which cause damage to the target cells. MB mediated PDT alleviates periodontitis through its antimicrobial effect and also inhibits the progression of

periodontitis by inducing apoptosis of over infiltrated macrophages [8]. The aim of this review is to determine the efficacy of methylene blue in antimicrobial photodynamic therapy (aPDT) for treatment of periodontitis.

AIM:

To answer the following PI(E)COS question.

In patients with periodontitis, what is the efficacy of methylene blue in Antimicrobial photodynamic therapy (aPDT), in terms of clinical attachment level (CAL)?

Where,

PARTICIPANTS/POPULATION(P) - Patients suffering from periodontitis

INTERVENTION(S), EXPOSURE(S)-Methylene blue mediated Antimicrobial photodynamic therapy (aPDT)

COMPARATOR(S)/CONTROL(C)-Non-surgical and/or surgical treatment alone for management of chronic or aggressive periodontitis.

OUTCOME (O) - Clinical, immunologic or microbiologic parameters.

STUDY DESIGN- In-vivo human randomized and/or controlled clinical trials.

PRIMARY OUTCOME- Alteration in clinical attachment level (CAL)

SECONDARY OUTCOME(S)-Probing pocket depth (PPD), Bleeding on probing (BOP), Gingival recession (GR), Gingival index (GI), plaque index (PI) and Microbiological or immunologic analysis.

OBJECTIVES

To systematically review the literature in order to produce a database of outcome variables that have been utilized for Clinical, immunologic or microbiologic parameters.

MATERIALS AND METHODS

PROTOCOL

A protocol was developed following the PRISMA (Preferred Reporting Items for Systematic Review and Meta Analyses) statement. This systematic review is registered on PROSPERO International prospective register of systematic reviews 2020 : CRD42020223338.

SEARCH STRATEGY

The following databases were thoroughly searched:

MEDLINE (NCBI PubMed and PMC), Scopus, Cochrane Central Register of Controlled Trials (CCRCT),ScienceDirect , Google Scholar ,EMBASE, EBSCO.

The following journals were hand searched: Journal of Clinical Periodontology, Journal of Periodontology, Journal of Indian Society of Periodontology, and Photobiology, Photo diagnosis and Photodynamic Therapy.

The following combinations of title, abstract, Medical Subject Heading Terms (MeSH) and keywords were used to search through the above-mentioned databases. (Photochemotherapy) AND (Periodontitis) AND (Methylene Blue) AND (Photosensitizing agents) AND (Periodontal pocket) AND (Photodynamic therapy).

STUDIES TO BE INCLUDED

1. Randomized Controlled Trials and/or Controlled Clinical Trials comparing the efficacy of Methylene blue in aPDT with non-surgical and/or surgical management of chronic or aggressive periodontitis.
2. Studies reporting at least one of the following parameters as an outcome variable: probing pocket depth, clinical attachment level, gingival recession, bleeding on probing, plaque index, gingival index, microbiological profile or immunological profile.
3. Studies with the follow up of at least 1 month after treatment.

STUDIES TO BE EXCLUDED

1. Randomized Controlled Trials and/or Controlled Clinical Trials comparing the efficacy of any dye other than methylene blue in aPDT with non-surgical and/or surgical management of chronic or aggressive periodontitis.
2. Narrative literature reviews, case reports, in vitro studies, in vivo animal studies, commentaries, interviews, updates, case series

Each study was assigned an exclusive Reference ID for easy identification and simplification of data collection procedure. The Reference ID was prepared with the initials of first author and alphabetic order. The Revised Cochrane Risk-of-Bias tool for Randomized trials, Version 2.0 (RoB 2) was used.

RESULTS

A full search from multiple databases resulted in 3267 articles. Relevant articles were identified by two independent reviewers, 3114 duplicates were removed. 153 articles were selected for full text evaluation after screening the title and abstracts. 97 articles of in vitro and animal studies were excluded. Only In-vivo human studies were included. 56 articles of in vivo human studies were found. By applying the inclusion criteria, 30 articles were excluded. The total articles fulfilling the inclusion criteria were 26. There were 2

articles that did not have full texts, so they were excluded. 24 articles fulfilled the criteria to be included in the current systematic review. The doses of the methylene blue used were 10mg/ml in six studies, 0.005 % in five studies, 0.01% in five studies,1 % in three studies, 0.3% in two studies, 0.2 mL in one study, 100 μM in one study and 100 μg/mL in one study. (TABLE 1) Clinical Parameters were measured in 23 studies which included clinical attachment level (CAL), probing pocket depth (PPD), plaque index (PI), gingival index (GI), bleeding on probing (BOP), gingival recession (GR) and only one study did not measure clinical parameters. Microbiological parameters were measured in 6 studies which included the detection of periodontal bacteria (*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Treponema denticola, Prevotella intermedia, Parvimonas micra*). Only four studies measured the immunological parameters which included the samples from Gingival Crevicular Fluid (GCF). (TABLE 2)
An overall assessment for the Risk of Bias showed a high risk for 6 studies, risk of some concerns for 4 studies while low risk of bias for 14 studies.

DISCUSSION

Classical concept of treatment for Periodontitis includes plaque control nonsurgical procedures followed by surgical therapy if needed [9]. Antimicrobial Photodynamic therapy (aPDT) is a new set of procedures that shew necessary as adjunctive periodontal treatment [10]. It uses many dyes such as Indocyanine green, Toluidine blue, Acridine orange, etc. Methylene blue dye exerts antimicrobial effect, by entering gram-negative bacteria through the porin-protein channels of the outer membrane which upon activation using a diode laser, releases oxidizing metabolites that have detrimental effects on lipopolysaccharide [11]. Twenty-four RCTs fulfilled the inclusion criteria with systemically healthy patients with chronic periodontitis and with a minimum follow up period of 1 month. The results of the present systematic review indicates that Methylene blue mediated aPDT produces statistically significant improvements in terms of outcomes variables such as CAL, PPD, PI, GI, BOP, and GR when compared with Scaling and root planning alone. In present systematic review, twenty studies compared Clinical attachment level and Twenty-two studies compared Probing pocket depth in the test groups (SRP+ aPDT) with the control group (SRP alone). One study [12] showed higher CAL gain and PPD (6 ± 0.4 and 5.5 ± 1.2 respectively) in the control group as compared to the test group at 3 months follow-up while [13] showed significant improvement in the test group as compared to control group. The mean CAL (mm) gain and PPD reduction from baseline to 6 months in (SRP and PDT-1% methylene blue solution group) was 2.55 ± 0.44 and 2.57 ± 0.53 respectively. However,[14] report that CAL gain and PPD (reduction did not improve statistically significant between the test and control groups at 6 months follow up. In present systematic review, eighteen studies compared Plaque index in the test groups (SRP+ aPDT) with the control group (SRP alone). [15] reported that the test group (31.5 ± 6.2) had a significantly lower plaque index than the control group (35.4 ± 4.7) at 180 days follow-up. [16] demonstrated that PI was significantly reduced at all-time points compared to baseline in both case and control group at 6 weeks, 3 months and 6 months follow-up. Similarly, [17] reported, at 6 weeks, the PI had significantly reduced from 88 ± 18.5% to 15 ± 12% in aPDT group and 83 ± 16% to 16 ± 7% in SRP groups. In present systematic review, only Seven studies compared Gingival index in the test groups (SRP+ aPDT) with the control group (SRP alone). These studies showed reduced the levels of gingival inflammation. The Reduction in GI scores were highest at 1 month, 3 months in the test group [13] which was similar to study by [18] at 6 months follow-up. [19] reported, no differences in GI were found between 1, 3 and 6 months between test and control groups which were similar to previous study [20] in which GI parameters indicated significant reductions from baseline to day 32 for all groups (SRP and laser, Laser, SRP alone and OHI group). Furthermore, eighteen studies compared Bleeding on probing in the test groups (SRP+ aPDT) with the control group (SRP alone) in present systematic review. [21] demonstrated no statistically significant differences in BOP reduction between treatment arms (aPDT + SRP GROUP -48% and 50%) and (SRP GROUP- 46% and 50 %) at week 6 and at week 12 respectively. [12] reported BOP was comparable among individuals that received SRP alone and SRP + aPDT. In one study, [22] stated significant reduction in the number of BoP-positive sites was detected in both the SRP + PDT (80%) and SRP groups (60%) at 3 months follow-up. In present systematic review, only five studies compared Gingival recession in the test groups (SRP+ aPDT) with the control group (SRP alone). Two studies were in favour of the test group. [23] showed significant improvement in GR was 0.5 ± 0.6 in test group at 6 months follow-up. Similarly, the increase in GR only reached a statistically significance in moderate pockets of the test group (36.73) after 6 months. [24] There was no statistically significant difference between the test group and control groups in three studies [14],[25], [26] In present systematic review, six studies measured Plaque samples and compared the test groups (SRP+ aPDT) with the control group (SRP alone). There was only one study [20] which supported the test group. The proportions of obligate anaerobes decreased notably in SRP+LASER group from 50.54 ± 27.29 to 16.36± 22.28. [27] carried out a study using aPDT on *Aggregatibacter actinomycetemcomitans* biofilm and stated bacterial

reduction of 99.85% in the group treated with aPDT and irradiated for 5 min. Their results were that the irradiation time exerts an influence on cell death. Considering immunological analysis in present systematic review, four studies evaluated and compared the test groups (SRP+ aPDT) with the control group (SRP alone). [17] assessed gingival crevicular fluid samples for TNF-α and IL-6 using enzyme linked immunosorbent assay (ELISA). IL-6 and TNF-α levels decreased significantly at 12 weeks after therapy in both the groups. [14] found a significant decrease between baseline and month 6 for C-reactive protein (CRP), serum amyloid A, fibrinogen, procalcitonin, and α2M in GCF levels. [28] showed a greater reduction of IL-1β expression in GCF 1 week and 1 month after aPDT therapy. These findings suggests that aPDT leads to an increase of the immunomodulatory activity of the tissue, by decreasing T lymphocytes stimulus and through the inactivation of important pro-inflammatory markers found in the periodontal disease, thus resulting in a decrease of the inflammatory cell number after treatment in patients with periodontitis. The role of aPDT on the profile of the inflammation mediators are described [29] , [30], [31] still, it is important to highlight that the clinical conditions such as time of performance and photosensitizer concentration, pH change, exudate presence and gingival fluid in the subgingival environment can influence the effectiveness of therapy [30]. Thus, the comparison between different studies is hindered by the vast number of protocols, such as various laser parameters, different photosensitizers concentrations, and by changes in periodontal conditions and periodontal treatments.

TABLE 1: METHODOLOGICAL CHARACTERISTICS OF STUDIES ON METHYLENE BLUE IN PHOTODYANAMIC THERAPY

SR.NO.	REF ID	TEST PATIENTS BASELINE (FOLLOW UP)/CONTROL PATIENTS BASELINE (FOLLOW UP)	INTERVENTION GROUP (METHYLENE BLUE)		CONTROL GROUP	FOLLOW UP
			DOSE	LASER PARAMETERS		
1.	SY (a)	10 (10)	0.005%	Gallium-Arsenide diode laser (BTL-2000 Prague, Check, Rep., BTL Co., Check Rep.) operating at a frequency of 5.0 Hz and delivering a 30-mW continuous wave output at 685 nm with a power density of 1.6 J/cm2	Oral hygiene instructions (OHI) Group	32 days
2.	MA(b)	45(45)/ 45(43)	0.01 %	670-nm non-thermal diode laser.	Scaling and root planning (SRP)	3 months
3.	GC (c)	15(15/15(13)	10mg/ml	Diode laser (Thera Lase—DMC, São Paulo, SP, Brazil) with a wavelength of 660 nm, a power output of 60 mW, and energy density of 129 J/cm2	Scaling and root planning (SRP)	3 months
4.	GN (d)	15(15) / 15 (12)	0.01%	Diode laser with a wavelength of 660 nm and 0.03 W power.	Scaling and root planning (SRP)	45 days, 3 and 6 months.
5.	MG(e)	28(28)	0.3%	Diode laser operating at 635 nm wavelength.	Scaling and root planning (SRP)	1 year

6.	MB(f)	22(22)	0.005%	Low power laser – AsGaAl (Photon Lase III – PL7336, 660 nm, 100 mW, 9 J, 90 seconds per site, 320 J/cm2 , diameter tip 600 µm. DMC, São Carlos –SP, Brazil)	Ultrasonic debridement	1,3 and 6 months
7.	VM (g)	28(27)	0.2 ml	Fiber optic cable to a diode laser (λ 670 nm, 280 mW of output power.	Sham treatment without activating the laser	3 and 6 months
8.	HD (h)	16(16)	10mg/ml	Diode laser HELBO Theralite laser (wavelength 660 nm and power output of 100 mW)	Oral hygiene instructions and calculus removal	2 weeks, 3 months and 6 months.
9.	BJ (i)	44 (40) / 44(40)	10 mg/ml	Diode laser (CNI Opto-electronics Tech. Co. Ltd, China) operating at 655 nm with a CW output power of 1W (CSP)	Scaling and root planning (SRP)	2 weeks,1,3 and 6 months.
10.	VC (j)	(18) / 19 (16)	0.01 %	Diode laser with wavelength of 660 nm, using an optic fibre tip into the periodontal pocket for 90 s and energy density of 90 J/cm^2, 40 mW power. (Laser Hand– MM Optics, S~ao Carlos, SP, Brazil).	Sham procedure	3,6 and 12 months.
11.	MC(k)	20(15)	10 mg/ml	Diode laser (Thera Lase—DMC, Sao Paulo, SP, Brazil) with ~ a wavelength of 660 nm, a power output of 60 mW, and an energy dose of 129 J/cm^2	Scaling and root planning (SRP)	3 months.
12.	MG(l)	26 (24) / 26 (24)	0.3%	A 635 nm diode laser	Sham treatment + SRP	1 and 4 years.
13.	SM(m)	24(24)	1%	The diode laser (DenLase; China Daheng Group, Inc. Beijing CHINA) was operated at a peak power of 5.0 W, with a pulse length of 200 µs and pulse interval of 200 µs (average power 1.0 W), using a 400 µm fiber-optic tip and a wavelength of 980 nm.	Scaling and root planning (SRP)	1, 3 and 6 months.
14.	PA(n)	28(28)	0.01%	Low power laser: 660 nm, 40 mW, 90 J/cm^2.	Saline solution was used for the subgingival irrigation.	3 and 12 months.

15.	SM(o)	24(24)	1%	980 nm Diode Laser (DenLase, the Diode Laser Therapy System, from China Daheng Group, Inc.)	Scaling and root planning (SRP)	1,3 and 6 momths
16.	RA (p)	58(55)/63(57)	0.01%	A low power (<225mW), continuous–wave diode laser (Periowave™, Ondine Biomedical, Vancouver, BC) operating at a red wavelength (670nm) over a 60–second pre–programmed treatment cycle.	Scaling and root planning (SRP)	6 and 12 weeks.
17.	FV(q)	23(23)/29(29)	0.005%	A diode laser of 670 nanometers at 150 milliwatts with optic fibre diameter 0.06 mm.	Scaling and root planning (SRP)	6 and 12 weeks.
18.	BA(r)	18(18)/18(18)	10 mg/ml	(Thera Lase DMC – Brazil), a wavelength of 660 nm, a power of 60 mW, and a fluency of 129 J/cm^2	Ultrasonic periodontal debridement + placebo pill	3 and 6 months
19.	FB(s)	6(6)/6(6)	10 mg/ml	Red laser (660 nm-40 mW)	Non-surgical periodontal therapy (NSPT)	30, 90 and 180 days.
20.	FA(t)	42(42)/41(41)	0.005%	Diode laser (660 nm) at 150 mW.	Scaling and root planing	1 and 3 months.
21.	LA (u)	30(30)/ (30(30)	100 µM	Red laser (Photon Lase III, DMC, São Carlos, Brazil) (660 nm, 100 mW)	Application of Methylene Blue	_____
22.	FK(v)	21(21)/21(21)	1%	670 nm diode laser	Scaling and root planing	3 and 6 months.
23.	AF(w)	20(20)/20(20)	0.005%	Diode laser (Therapy – Plus, DMC®, São Carlos, Brazil) red (wavelength of 660 nm, power 100 mW, spot size 600 µm and energy density of 60 J/cm^2) and infrared (wavelength of 808 nm, power 2500 mW, spot size 600 µm and energy density of 140 J/cm^2)	LLLT +SRP	4 and 12 weeks
24.	ND(x)	25(25)/25(25)	100 µg/mL	Diode laser (DX61, Konftec, Taiwan) at wavelength of 660 nm, power of 150 mW	Scaling and root planing	6 weeks, 3 and 6 months

TABLE 2: SUMMARY OF PRIMARY AND ADDITIONAL OUTCOME

	CLINICAL PARAMETERS						MICROBIOLOGICAL PARAMETERS	IMMUNOLOGICAL PARAMETERS
REF ID	CLINICAL ATTACHMENT LEVEL (CAL) (Baseline/follow-up)	PROBING POCKET DEPTH (PPD) (Baseline/follow-up)	PLAQUE INDEX (PI) (Baseline/follow-up)	GINGIVAL IDEX (GI) (Baseline/follow-up)	BLEEDING ON PROBING (BOP) (Baseline/follow-up)	GINGIVAL RECESSION (GR) (Baseline/follow-up)	(Baseline/ follow-up)	(Baseline/follow-up)
SY (a)	___	Group SRP and Laser-(0.66) Group Laser-(0.23) Group SRP-(0.49) Group OHI (0.19)	Group SRP and Laser-(1.60) Group Laser-(0.71) Group SRP-(1.57) Group OHI (0.64)	Group SRP and Laser-(1.03) Group Laser-(0.60) Group SRP-(1.17) Group OHI (0.53)	Group SRP and Laser-(60) Group Laser-(17) Group SRP-(50) Group OHI (20)	_____	Total Viable counts (* 10^3 CFU/ml) of Subgingival samples at baseline and 3 weeks after treatment. Group SRP and Laser-(19.08/15.31) Group Laser-(15.69/15.89) Group SRP-(10.57/8.41) Group OHI (12.60/11.04)	_____
MA (b)	Group SRP-(4.66/4.10) Group SRP + Doxy (3.9/3.41) Group SRP+ PDT- (4.33/3.87)	Group SRP-(3.24/2.64) Group SRP + Doxy (3.26/2.82) Group SRP+ PDT- (3.00/2.5556	Group SRP-(0.86/0.59) Group SRP + Doxy (0.88/0.52) Group SRP+ PDT- (0.90/0.)	_____	Group SRP-(0.72/0.43) Group SRP + Doxy (0.87/0.62) Group SRP+ PDT- (0.72/0.54)	_____	_____	_____
GC (c)	Group PDT+SRP-(11.93/10.50) Group SRP-(10.81/10.30)	Group PDT+SRP-(6.20/14.03) Group SRP-(5.44/14.30)	(42.02/19.21)	_____	Group PDT+SRP-(100/20.22) Group SRP-(100/60)	_____	_____	_____
GN (d)	Group SRP-(3.7/3.1,3.3,3.6) Group SRP+PDT-(4.0/2.9,2.8,2.6)	Group SRP-(3.3/2.6,2.8,3.0) Group SRP+PDT-(3.5/2.4,2.3,2.1)	Group SRP-(81.9/29.8,28.4,45.1) Group SRP+PDT-(83.0/24.3,27.8,29.9)	_____	Group SRP-(89.0/35.4,45.1,72.9) Group SRP+PDT-(79.2/16.0,16.7,18.7)	Group SRP-(0.5/0.5,0.5,0.6) Group SRP+PDT-(0.4/0.5,0.5,0.5)	The presence of *Porphyromonas gingivalis (Pg), A. actinomycetemcomitans (AA),* and *Tannerella forsythia* was	_____

							evaluated. Patients in both SRP groups, isolated or associated with PDT, showed statistically significant reductions of Pg, Aa, *T.forsythia* amounts by the 6th month.	
MG (e)	Group Sham+SRP-((5.6/4.8) Group Laser + SRP-(5.6/3.1)	Group Sham+SRP-(4.9/4.0) Group Laser + SRP-(5.1/2.1)	_____	_____	Group Sham+SRP-(68.9/37.0) Group Laser + SRP-(69.4/3.8)	_____	_____	_____
MB (f)	NS	NS	(18.83/12.50, 16.68,13.60)	(37.48/30.73, 27.86,25.37)	Test group-(61.58/46.16, 40.67,36.73) Control group-(62.23/47.41, 42.21,38.49)	Test group-(1.5/2,1.8,2) Control group-(2/1.9,2,1.8)	_____	_____
VM (g)	Group A (7/4,4.7) Group B (7.9/4,4.) Group C (7.6/4.7,4.6)	Group A (5.9/2.9,3.1) Group B (6.3/2.8,2.9) Group C (7.6/4.7,4.6)	Group A (17/8,11) Group B (19/11,12) Group C (19/12,12)	Group A (22/11,14) Group B (22/11,10) Group C (20/14,13)	Group A (16/7,10) Group B (20/9,7) Group C (15/8,10)	_____	Quantitative real-time PCR was performed to detect and quantify six specific bacteria (*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Treponema denticola, Prevotella intermedia, Parvimonas micra)* using species-specific primers. Detection frequencies and frequencies of sites with counts >100.000 cells/ml of the studied microorganisms did not change between	GCF levels of 20 different biomarkers were determined using a multiplex fluorescent bead-based immunoassay and the Bio-Plex 200 suspension array system (BioRad Laboratories, Hercules, CA, USA) A significant decrease was observed between baseline and 6

							baseline and 3 or 6 months in any of the three treatment groups.	months after treatment for CRP, serum amyloid A, fibrinogen, procalcitonin, and α2M. When looking at the groups separately, CRP was significantly lower at month 6 only after treatment according to protocol A.
HD (h)	Group 1 (3.8/3.4,3.3,3.6) Group 2 (6.7/6.8,6.8,8.1)	Group 1 (3.3/2.8,2.5,2.9) Group 2 (5.8/4.7,4.5,6.5)	____	_____	_____	_____	_____	_____
BJ (i)	Test Group- (6.5/5.1,4.0,4.0) Control Group- (6.0/5.1,4.4,4.5)	Test Group- (5.7/4.0,3.3,3.0) Control Group- (5.5/4.7,3.9,4.0)	Test Group- (2.0/0.8,0.5,0.5,1.0) Control Group- (1.2/1.0,0.5,0.5,0.5)	Test Group (2.0/1.0,0.75, 0.8,1.0) Control Group- (2.2/1.5,1.0,1.0,1.5)	_____	Test Group- (1.0/1.0,1.0,1.0) Control Group- (1.0/1.0,1.0,1.0)	_____	_____
VC (j)	Test group- (5.56/4.61,4.44,4.60) Control group- (5.87/4.78,4.78,4.33)	Test group- (4.79/3.70,3.41,3.55) Control group- (4.87/3.39,3.48,3.23)	Test group- (18.05/12.50, 16.67,12.50) Control group- (18.75/10.93, 09.33,6.25)	_____	Test group- (58.33/19.44, 31.94,23.61 Control group- (42.18/17.18, 23.43,15.63)	_____	Bacterial detection and quantification were performed using a quantitative real-time PCR (qPCR) TaqMan system (Applied Biosystem, Foster City, CA, USA) for species: *A. actinomycetemcomitans, P. gingivalis, Treponema denticola (T.*	_____

							denticola) and *Tannerella forsythia (T. forsythia).* There was a significant association between group and presence of *Porphyromonas gingivalis* at 12 months (p = 0.02), as 88.9% of the test subjects harboured these bacteria, as compared to 50% of the control subjects. There were no differences between groups at any time of study, for any of the bacterial species.	
MC (k)	Group SRP+PDT (11.9/10.6) Group SRP (10.5/10.2)	Group SRP+PDT (6.3/3.9) Group SRP (5.5/3.9)	Group SRP+PDT (0/27) Group SRP (0/13)	_____	Group SRP+PDT (100/20) Group SRP (100/40)	_____	Microbiological assays, primers, and reaction templates were performed to determine the absolute quantification of *Aggregatibacter Actinomycetemcomitans and Porphyromonas gingivalis*. Real-time PCR analysis revealed that, at baseline, the concentration of *A. actinomycetemcomitans* was similar in both groups However, at SRP + PDT treated sites,	_____ _____

							statistically significant reductions were observed in the concentration of this pathogen on the 3rd and 7th days after therapy, although no significant changes were observed in SRP treated sites throughout the study.	
MG (l)	Group Sham treatment + SRP-(5.6/4.8,5.5) Group PAPD + SRP (5.6/3.1,2.2)	Group Sham treatment + SRP-(4.9/4.0,4.3) Group PAPD + SRP (5.1/2.1,1.2)	____	_____	Group Sham treatment + SRP-(68.9/37.0,45.8) Group PAPD + SRP (69.4/3.8,4.2)	_____	_____	_____
SM (m)	Group SRP-(6.63/5.84,4.83,4.00) Group SRP +PDT (6.59/,5.71,4.84,4.04)	Group SRP-(6.16/5.22,4.43,3.65) Group SRP +PDT (6.13/5.09,4.28,3.57)	Group SRP-(2.49/1.21,0.76,1.60) Group SRP +PDT (2.54/1.21,0.84,1.73)	Group SRP-(2.42/1.57,0.91,0.63) Group SRP +PDT (2.33/1.51,0.94, 0.54)	_____	_____	_____	_____
PA (n)	Group PDT (5.54/4.5,4.61) Group Control (5.5/4.09,3.94)	Group PDT (4.69/3.58,3.62) Group Control (4.71/3.19,3.25)	Group PDT (53.5/17.8,17.85) Group Control (44.6/17.8,16.07)	_____	Group PDT (17.85/10.71, 12.5) Group Control (14.28/10.51, 5.35)	_____	_____	Samples were analyzed in duplicate using Luminex Performance Assay Kit 9 to determine IL-1β, IL-1α, IL-8, IL-10, IL-4, IL1-RA, TNF-α, VEGF, IFN-γ, and FGF levels.
SM (o)	Group SRP (6.63/5.84,4.83,4) Group SRP+ aPDT(6.59/5.71,4.84,4.04) Group SRP + aPDT + LLLT (6.76/5.67,4.57,3.69)	Group SRP (6.16/5.22,4.43,3.65) Group SRP+ aPDT(6.13/5.09,4.28,3.57) Group SRP + aPDT + LLLT (6.36/5.24,4.12,3.23)	Group SRP (2.49/1.21,0.76,1.60) Group SRP+ aPDT(2.54/1.21,0.84,1.73) Group SRP + aPDT + LLLT (2.53/0.75,0.97,2.27)	Group SRP (2.42/1.57,0.91,0.63) Group SRP+ aPDT(2.33/1.51,0.94,0.54) Group SRP + aPDT + LLLT (2.36/1.52,0.77,0.19)	_____	_____	_____	_____
RA (p)	Group aPDT + SRP	Group aPDT + SRP	_____	_____	Group aPDT + SRP	_____	_____	_____

	(2349/-0.69, -0.71) Group SRP (2463/-0.50, -0.54)	(2352/-0.82, -0.85) Group SRP (2460/-0.69, -0.68)			(1706/48%, 50%) Group SRP (1552/46%, 50%)			
FV (q)	Group SRP + aPDT (5.0/4.5,4.4) Group SRP (5.5/5.2,5.1)	Group SRP + aPDT (4.0/2.8,2.6) Group SRP (4.2/3.2,3.1)	Group SRP + aPDT (88.2/15.5,12.6) Group SRP (82.7/16.2,11.8)	_____	Group SRP + aPDT (85.4/16.8,13.6) Group SRP (82.7/13.0,11.3)	_____	_____ _	Gingival crevicular fluid samples for TNF-α (TNF-α Human ELISA kit, Abcam, UK) and IL-6 (Human interleukin-6, ELISA Kit, Abcam, UK) were assessed using enzyme linked immunosorbent assay (ELISA). . Patients in the aPDT and SRP groups showed comparable levels of TNF-α and IL-6 at baseline. IL-6 and TNF-α levels decreased significantly at 12 weeks after therapy in both the groups. Intergroup comparison showed significant difference for TNF-α

								and IL-6 levels for aPDT group at 12-week follow-up.
BA (r)	Group UPD (7.1/5.1,4.8) Group UPD+aPDT (7.3/4.8,4.9) Group UPD + CLM (7.5,4.4,4.5) Group UPD + CLM + aPDT (6.7/3.6,3.7)	Group UPD (7.1/5.0,4.6) Group UPD+aPDT (7.2/4.4,4.6) Group UPD + CLM (6.8/3.7,3.7) Group UPD + CLM + aPDT (6.7/3.6,3.5)	Group CLM (57/30) Group UPD (65/19)	_____	Group UPD (100/33.3,38.8) Group UPD+aPDT (100/33.3,33.3) Group UPD + CLM (100/11.1,16.6) Group UPD + CLM + aPDT (100/22.2,16.6)	Group UPD (0.0/0.1,0.2) Group UPD+aPDT (0.1/0.4,0.3) Group UPD + CLM (0.7/0.7,0.7) Group UPD + CLM + aPDT (0/0,0.2)	_____	_____ _____
FB (s)	Group aPDT (2.9/2.2,2.3,2.3) Group NSPT (3.1/2.1,2.2,2.3)	Group aPDT (2.3/1.6,1.7,1.8) Group NSPT (2.9/2.0,2.1,2.2)	Group aPDT (31.4/25.2,35.4,31.5) Group NSPT (32.4/37,36.1, 35.4)	_____	Group aPDT (43.9/18.7,22.4,18.7) Group NSPT (55.5/ 21.4,31.7, 32.1)	_____	_____ _____	HbA1c and fructosamine, there were no significant differences between or within the groups at any evaluation period.
FA (t)	Group 1 SRP (7.1/6.3,6) SRP+aPDT (7.4/6.4,6.2) Group 2 SRP (7.2/5.2,4.9) SRP + aPDT (7.1/5,5.2)	Group 1 SRP (6.1/5.4,5.5) SRP+aPDT (6.4/5.7,5.8) Group 2 SRP (6.6/4.4,4.1) SRP + aPDT (6.4/4.6.4.2)	Group 1 SRP (52.6/34.9,38.5) SRP+aPDT (55.2/30.3,35.2) Group 2 SRP (57.3/20.2,23.5) SRP + aPDT (54.8/21.6.22.3)	_____	Group 1 SRP (35.3/30.6,32.7) SRP+aPDT (31.6/28.7,28.2) Group 2 SRP (61.2/17.3,20.3) SRP + aPDT (65.4/19.4,21.6)	_____	_____ ___	_____ _____
LA (u)	_____	_____	_____	_____	_____	_____	The samples were used to determine the number of CFU (Colony Forming Unit). the growth pattern of the bacterial colonies in	_____ _____

							the Petri dishes, verified that in MB1, MB3, and MB5 groups, there was no bacterial reduction and the growth pattern of the colonies remained the same before and after irradiation. In the groups treated with MBS vehicle, the pockets irradiated for 1 and 3 min (MBS1 and MBS3, respectively) did not present a significant reduction in the number of CFU/mL. However, there was a change in the growth pattern of the colonies.	
FK (v)	Group SRP (5.29/4.14,4.05) Group Diode laser (5.30/4.32,4.23) Group PDT (5.49/4.49,4.45)	Group SRP (4.80/3.26,3.14) Group Diode laser (4.82/3.12,3.19) Group PDT (4.76/3.18,3.10)	Group SRP (77.6/26.6,21) Group Diode laser (85.5/26.6,30.6) Group PDT (82.9/31.6,27.6)	_____	Group SRP (81.9/21.9,13.3) Group Diode laser (80/18.4,14.6) Group PDT (79/18.1,15.9)	_____	_____	_____
AF (w)	Group aPDT (4.19/3.74,3.51) Group LLLT (4.16/3.62,3.44)	Group aPDT (3.04/2.39,2.36) Group LLLT (3.01/2.28,2.23)	_____	_____	Group aPDT (27.37/11,5.89) Group LLLT (26.16/8.89,5.05)	Group aPDT (1.14/1.35,1.15) Group LLLT (1.15/1.34,1.20)	_____	_____
ND (x)	_____	Group SRP (6.92/5,3.88,3.20) Group aPDT + SRP (7.04/5.40,3.08,2.64)	Group SRP (6.96/4.68,3.72,3) Group aPDT + SRP (6.48/4.48,3.84,2.92)	Group SRP (6.92/5.04,4,3.04) Group aPDT + SRP (6.76/4.56,3.64,2.88)	_____	_____	_____	_____

CONCLUSION

Methylene blue mediated aPDT can influence several clinical parameters such as CAL, PPD, PI, GI, BOP and GR compared to SRP alone in the management of periodontitis. Within the limitations of these studies, present systematic review concludes methylene blue mediated aPDT can be a treatment modality for the management of periodontitis.

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