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Comparative Evaluation of Riboflavin and Methylene Blue Mediated Photodynamic Therapy on Periodontal Parameters in Orthodontic Patients: A Split-Mouth Randomized Controlled Trial

Javed Sodawala [1*], Ranjit Kamble [1], Samkit Bothra[2], Fatema Sodawala [3], Piyush Khandelwal [4], Tanusha Mahobia [5]

[1] Professor, Department of Orthodontics and Dentofacial Orthopedics, Sharad Pawar Dental College, Datta Meghe Institute of Medical Sciences, Sawangi, Wardha, Maharashtra, India
[2] Assistant Professor, Department of Orthodontics and Dentofacial Orthopaedics, Chhattisgarh Dental College and Research Institute, Rajnandgaon, Chhattisgarh, India
[3] Associate Professor, Department of Prosthodontics, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh, India
[4] Professor, Department of Orthodontics and Dentofacial Orthopaedics, Chhattisgarh Dental College and Research Institute, Rajnandgaon, Chhattisgarh, India
[5] Associate Professor, Department of Orthodontics and Dentofacial Orthopaedics, Chhattisgarh Dental College and Research Institute, Rajnandgaon, Chhattisgarh, India

***Corresponding author:** Javed Sodawala

Address: Department of Orthodontics and Dentofacial Orthopedics, Sharad Pawar Dental College, Datta Meghe Institute of Medical Sciences, Sawangi , Wardha - 442001, Maharashtra, India.

E-mail: drjavedazher@gmail.com

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Abstract

Aim
This study aimed to evaluate the antimicrobial effectiveness of riboflavin-mediated photodynamic therapy (PDT) on periodontal health in patients undergoing fixed orthodontic treatment and to compare its effects with methylene blue-mediated PDT.

Methods
A split-mouth randomized clinical trial was conducted on 40 patients undergoing fixed orthodontic therapy, comprising a total of 160 quadrants. The quadrants were randomly allocated into four groups: riboflavin PDT (RB), methylene blue PDT (MB), control for riboflavin (CR), and control for methylene blue (CM). Photodynamic therapy was applied in the RB and MB groups at baseline and on days 7, 14, and 21. Clinical periodontal parameters—including plaque index (PI), gingival index (GI), probing depth (PD), and bleeding on probing (BOP)—as well as microbial colony-forming units (CFU) were recorded at baseline and after 28 days. Statistical analysis included paired t-tests and Wilcoxon signed-rank tests for intragroup comparisons, and ANOVA with Tukey post hoc tests, along with Kruskal-Wallis and Mann-Whitney U tests for intergroup comparisons. The significance level was set at $P < 0.05$.

Results
All enrolled patients completed the study with no loss to follow-up. Both experimental groups showed significant reductions in PI, GI, PD, BOP, and microbial CFU from baseline to follow-up ($P<0.001$), with the MB group demonstrating greater improvements than the RB group. Control groups showed no significant changes in most parameters except for a slight reduction in BOP. Intergroup comparisons revealed no baseline differences ($P=0.833$), while at follow-up significant differences were observed among groups for PI, GI, BOP, and CFU ($P<0.05$). Additionally, PD was significantly lower in the MB group compared with both control groups ($P<0.001$). Reliability analysis showed excellent agreement for PI, GI, and BOP (ICC>0.90) and good agreement for PD and CFU (ICC>0.75).

Conclusion
Although methylene blue PDT showed superior outcomes, riboflavin PDT also significantly improved periodontal health and reduced microbial load in orthodontic patients. Considering its effectiveness and the advantage of not requiring

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specialized laser equipment, riboflavin PDT may serve as a practical alternative for clinical use, particularly in settings with limited resources.
Keywords: Methylene blue, Orthodontics, Periodontal health, Photodynamic therapy, Riboflavin.

Background

Orthodontic treatment, frequently initiated during childhood, enhances oral function and aesthetics, thereby improving patients' quality of life. Nevertheless, fixed orthodontic appliances often exacerbate periodontal inflammation due to biofilm buildup and the ensuing challenge of effective plaque control, potentially leading to gingivitis and progressive periodontal breakdown (1) Oral biofilm, a dynamic and complex community comprising diverse microbial species, is the principal etiologic factor in caries and periodontal disease (2).Photodynamic therapy (PDT) leverages a photosensitizer, light of a specific wavelength, and oxygen to generate reactive oxygen species (ROS), inducing oxidative damage in target microbes. PDT operates via two primary mechanisms: the type I pathway involves electron or hydrogen transfer from the excited photosensitizer to surrounding substrates, generating free radicals such as superoxide and hydroxyl radicals. The type II pathway involves energy transfer to molecular oxygen, producing singlet oxygen, a highly reactive species that causes oxidative disruption (3).

Since its first application in 1900, photodynamic therapy (PDT) has evolved into a non-invasive adjunct for managing oral infections (4). Commonly used synthetic photosensitizers—methylene blue, toluidine blue, and rose bengal—are typically activated by diode or gas lasers. Methylene blue is the most widely used photosensitizer for laser-based PDT, primarily exerting its antimicrobial effect via the type II photochemical pathway, which generates singlet oxygen and induces oxidative damage (1,5–8). However, it is often associated with mucosal staining, localized thermal effects, patient discomfort, and potential cytotoxicity to host tissues. Consequently, methylene blue-based PDT has not gained widespread adoption, as the requirement for expensive laser systems and specialized training puts it beyond the reach of many dental practices. These constraints highlight the need for a more accessible and cost-effective photodynamic approach.

Riboflavin (vitamin B2) serves as a natural photosensitizer, absorbing strongly in the blue spectrum (450–490 nm), and can be activated by compact, inexpensive light-emitting diode (LED) devices routinely available in dental clinics. It offers a compelling alternative, as it can be paired with low-cost, portable LED devices, has minimal staining, avoids thermal damage, and exhibits minimal host cytotoxicity, making it well-suited for routine use in fixed orthodontic patients (9). Previous studies have demonstrated riboflavin's antibiofilm and antimicrobial properties, but its clinical efficacy in orthodontic patients remains untested (10–12).

This randomized clinical trial was designed to compare periodontal health outcomes in fixed orthodontic patients treated with LED-activated riboflavin (RB) PDT versus laser-activated methylene blue (MB) PDT. The null hypothesis (H_o) was that there would be no difference between the two intervention groups in plaque index (PI), gingival index (GI), probing depth (PD), bleeding on probing (BOP), or colony-forming units (CFU) after four weeks (T1).

Methods

Study Design and Pre-clinical Trial Preparation

A split-mouth design was employed, allowing each patient to serve as their own control, thereby neutralizing inter-individual differences in oral hygiene practices, salivary composition, immune status, and baseline periodontal health. By applying experimental and control interventions to contralateral quadrants within the same mouth, we ensured that anatomical, behavioral, and systemic confounders remained constant, enhancing internal validity and sensitivity to detect true treatment effects. This approach also increased statistical power without requiring a larger cohort, permitting direct within-subject comparisons under identical clinical conditions and reducing variability arising from inter-subject heterogeneity.

This single-center, split-mouth, single-masked randomized clinical trial was prospectively registered at the Clinical Trials Registry of India (CTRI/2024/03/064860) and approved by the Institutional Ethics Committee of CDCRI (DEAN/ETHICSCOMMITTEE/ORTHO-03/2022). All the procedures conformed to the Declaration of Helsinki and to the ICMR's Guidelines for Biomedical and Health Research Involving Human Participants (2017) and followed CONSORT guidelines (Figure 1).

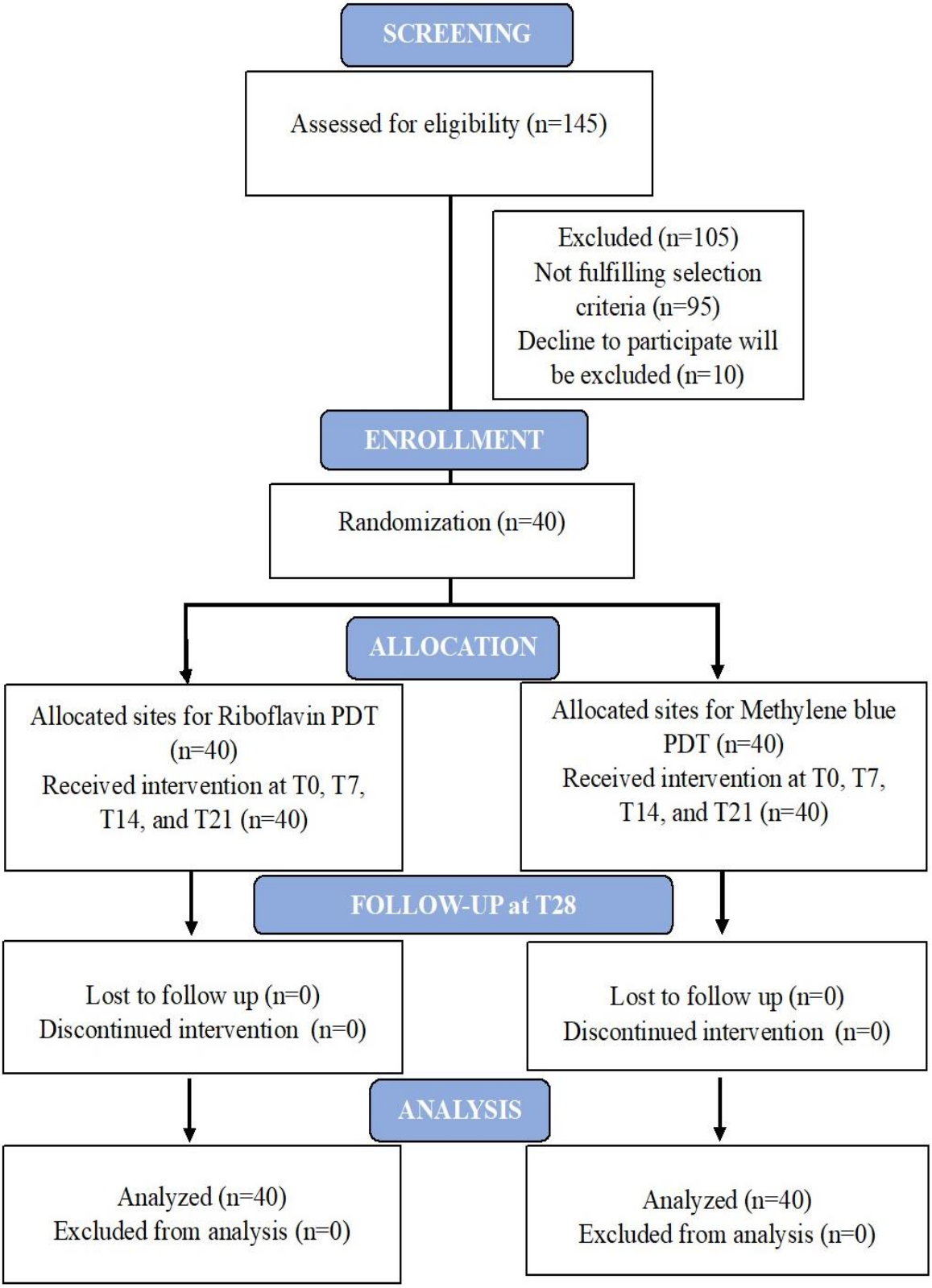

Figure1: CONSORT flow diagram of participant enrollment, allocation, follow-up, and analysis

Sample size estimation was performed a priori using G*Power 3.1.9.2 (Franz Faul Universität, Kiel, Germany). Assuming an effect size of 0.92 based on a pilot study, α=0.05, and power (1–β)=0.95 for a two-sided t-test, 32 quadrants per arm were required. Allowing 20% for attrition yielded 38.4 quadrants, rounded to 40. With four quadrant-level arms (riboflavin PDT (RB), riboflavin control (CR), methylene blue PDT(MB), methylene blue control (CM)), the total sample comprised 160 quadrants in 40 patients.

Forty orthodontic patients (aged 12–25 years) undergoing fixed appliance treatment for ≥6 months, with fully erupted permanent dentition (excluding third molars), minimal crowding (≤3 mm), and the ability to perform standardized brushing were recruited after written informed consent was obtained (self or parent/guardian). Exclusion criteria included systemic or pharmacological factors affecting the gingiva, adverse oral habits (such as smoking, tobacco use, nail-biting), carious or restored index teeth, periodontal therapy in the preceding six months, and pregnancy or lactation.

All the participants received a standardized fluoride toothpaste (Amflor toothpaste, Group Pharmaceuticals LTD, Mumbai, Maharashtra, India) and toothbrush(Colgate SlimSoft Ortho Toothbrush, Colgate-Palmolive Ltd. Sri City, Andhra Pradesh, India) along with oral hygiene instructions 15 days before baseline measurements (T0) to minimize variability.

Randomization was carried out at the quadrant level by an independent statistician using computer-generated blocks of four, with assignments concealed in sequentially numbered opaque envelopes. Each patient's four quadrants were allocated to one of four sequences, ensuring that each quadrant experienced one active PDT and one corresponding control condition. Due to the nature of the intervention, blinding of participants and treatment providers was not feasible. However, all clinical and microbiological assessments were performed by a single calibrated examiner blinded to the allocation.

To minimize bias during data analysis, the data operator responsible for statistical evaluation was blinded to group allocation throughout the study.

Photodynamic Therapy Procedure

At the initiation of orthodontic treatment, patients were explicitly instructed to refrain from consuming hard or sticky foods, as such dietary items may compromise the structural integrity of the fixed orthodontic appliances or impede the maintenance of optimal oral hygiene.

Photodynamic therapy was delivered at T0, and at 7 (T7), 14 (T14), and 21 days (T21). For riboflavin PDT, a 0.5% w/v solution (5 mg/mL; Hollywood Secrets, Goa, India) was prepared in sterile 0.9% saline, filtered, and stored at 4°C in amber vials for up to 7 days. Sectional metal trays were positioned around the control site to shield adjacent tissues and prevent off-target light exposure. The solution was injected into the gingival sulcus using a 31-gauge needle until the pocket was filled. It was then allowed to dwell for five minutes and gently rinsed with saline.

A continuous-wave dental LED (Waldent Innovations Pvt. Ltd., Chhatarpur, New Delhi, India; wavelength = 480 nm, intensity = 1500 mW/cm²) was held 2 mm from the tissue and activated for 30 s per site, delivering 45 J/cm² per exposure. Both facial and lingual surfaces were irradiated at each site, with one irradiation per timepoint (four total exposures from T0 to T21), for a cumulative fluence of 180 J/cm² (Figure 2a-d).

For methylene blue PDT, a 1% w/v solution (10 mg/mL; Labogens Fine Chem, Ludhiana, India) in distilled water was prepared, filtered, and stored at 4°C in amber vials for up to 7 days. Sectional metal trays were positioned around the control site to shield adjacent tissues and prevent off-target light exposure. The MB solution, was delivered into the gingival sulcus using a 31- gauge needle in a vertical orientation. i.e. , perpendicular to the gingival margin, until the sulcus was filled. This ensured uniform distribution within the sulcus. The solution was allowed to dwell for five minutes before gentle saline irrigation.

The activation was performed with a diode laser (Einstein DL, DC International LLC, FL, USA; wavelength = 670 nm, Power = 150 mW) via a 300-µm fiber tip in continuous mode (pulse length of 0.05 ms, a pulse interval of 0.02 ms) with each site irradiated for 30 s per session, delivering 22 J/cm² per exposure. Both facial and lingual surfaces were irradiated at each site, with one irradiation per timepoint (four total exposures from T0 to T21), for a cumulative fluence of 88 J/cm² (Figure 3a-c)

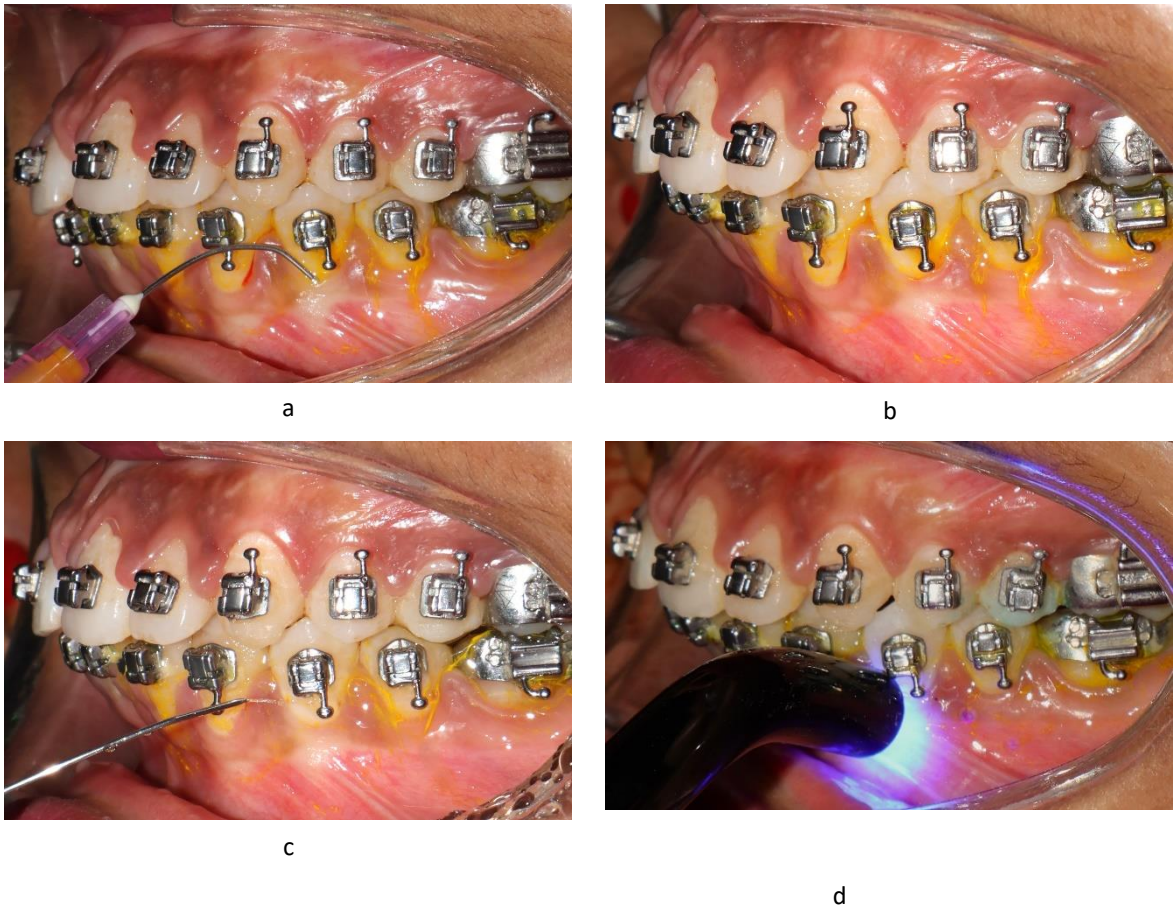

Figure 2. Riboflavin photodynamic therapy procedure.
a: Injection of 0.5% riboflavin solution into the gingival sulcus.
b: Clinical appearance immediately after applying 0.5% riboflavin solution.
c: Gentle saline rinse following a 5-minute dwell time.
d: A continuous-wave dental LED held 2 mm from the tissue and activated for 30 seconds per site to initiate photodynamic activation.

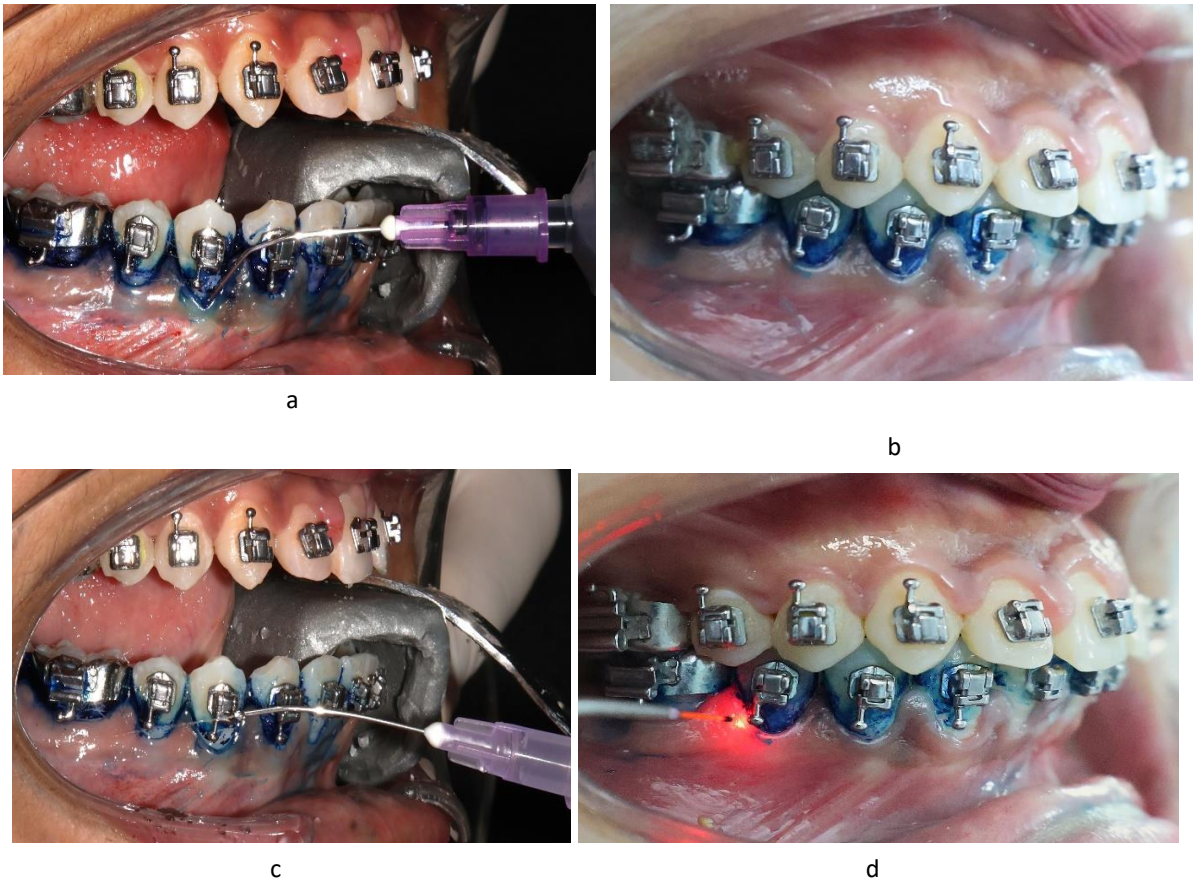

Figure 3. Methylene blue photodynamic therapy procedure.
a: Injection of 1% methylene blue photosensitizer solution into the gingival sulcus.
b: Clinical appearance immediately after applying 1% Methylene blue solution.
c: Gentle saline irrigation.
d: Photodynamic activation performed using a diode laser in continuous mode.

Microbial Sample Collection and Culture

Nutrient agar was prepared by dissolving 2.8 g of powder (TM media, Titan Biotech Ltd., Bhiwadi, Rajasthan, India) in 100 mL of distilled water. The mixture was then autoclaved at 121°C for 15 min and cooled to ~45°C before being poured. Under a laminar-flow hood, ~20 mL of agar was dispensed into sterile Petri dishes; the plates were allowed to solidify at room temperature. At baseline (T0) and on day 28 (T1), supragingival plaque was swabbed from four quadrants, collected from areas surrounding the orthodontic brackets, using sterile cotton-tipped applicators to avoid contamination with saliva or blood. The samples were inoculated on nutrient agar media plates using the direct swab culture technique. The plates (Teknik Industrial Solutions incubator, Pune, India) were incubated aerobically at 37±1°C for 24 h. Colonies were enumerated with a digital counter (Lab-Care Diagnostics Pvt. Ltd., Mumbai, India) and reported as CFU/mL of suspension to determine the overall viable bacterial load.

Assessment of Periodontal Health

Plaque index (PI), gingival index (GI), probing depth (PD), and bleeding on probing (BOP) were recorded at baseline (T0) and on day 28 (T1). In each quadrant, one permanent central incisor, canine, and first molar were selected as the index

teeth. For each parameter, the mean score of the three index teeth per quadrant was used for analysis. PI was scored by the Silness & Löe method (13) on four surfaces (mesiofacial, midfacial, distofacial, and lingual) of each index tooth. GI followed the Löe & Silness scale (14), with assessments at the distofacial papilla, facial margin, mesiofacial papilla, and lingual margin of each index tooth, providing a validated measure of gingival inflammation in orthodontic patients. PD was recorded at six sites per tooth (buccomesial, buccal, buccodistal, distolingual, lingual, and mesiolingual) using a UNC-15 periodontal probe with controlled force. BOP was assessed using the modified sulcular bleeding index (mSBI) of Mombelli et al. (15), scoring each site from 0 to 3 based on bleeding within 30 s of probing.

Statistical Analysis

Data were entered into Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA) and imported into SPSS 21.0 (IBM Corp., Armonk, NY, USA) for analysis. Descriptive data were expressed as means and standard deviations. Normality was checked using the Shapiro-Wilk test. An independent t-test was performed to compare the mean age between male and female participants. Intragroup comparisons regarding study parameters were performed using a paired t-test or Wilcoxon's signed-rank test, depending on the normality of data distribution. Intergroup comparisons regarding study parameters were performed using one-way ANOVA followed by post hoc Tukey tests or a Kruskal-Wallis test followed by a Mann-Whitney U test, depending on the normality of data distribution. Intra-examiner reliability for the study parameters was assessed by repeating measurements in 15% of the participants and calculating the intraclass correlation coefficient (ICC). The level of significance was set at 0.05.

Results

All the enrolled patients completed the study, and no participants were lost to follow-up. The mean age of the patients was 19.25±3.04 years, and an independent t-test confirmed that there was no significant difference in gender (P=0.11) (Table 1). Intragroup comparisons showed that both experimental groups experienced statistically significant reductions in all measured parameters from baseline (T0) to follow-up (T1). Specifically, in the RB group, PI decreased by 0.26, GI by 0.24, PD by 0.17 mm, and BOP by 0.45—all with P<0.001, while CFU dropped by 17.45×10^3/mL (P<0.001). In the MB group, reductions were even more pronounced with PI decreasing by 0.39, GI by 0.44, PD by 0.24 mm, and BOP by 0.75 (all P<0.001), and microbial CFU declining by 36.45×10^3/mL (P<0.001). In contrast, within the control groups, no significant differences were observed at T1 for most parameters, except for BOP, which decreased by 0.19 in the CR group and by 0.18 in the CM group (Table 2).

Intergroup comparisons revealed no significant differences at baseline (T0; P=0.833); however, at T1, significant differences emerged between the study groups for PI, GI, BOP, and CFU, with all intergroup differences reaching significance (P<0.05) except between the CM and CR groups (P>0.05). Additionally, significant differences in PD were found between the MB group and both control groups (MB vs. CM and MB vs. CR; P<0.001) (Table 3). Intraclass Correlation Coefficient (ICC) for PI, GI, and BOP was >0.90, indicating excellent reliability. ICC for PD and CFU was >0.75, indicating good reliability (Table 4).

Table 1: Descriptive statistics for age (in years) and gender

Gender	N	Minimum	Maximum	Mean	SD	P value
Female	23	13	24	19.91	2.97	0.11
Male	17	14	25	18.35	2.98	
Total	40	13	25	19.25	3.04	

Independent t-test, P>0.05=no significant difference

Table 2: Intragroup comparisons of different study parameters

Parameter	Group	T_0 (Mean ± SD)	T_1 (Mean ± SD)	P-value
Plaque index (PI)	RB	1.56±0.20	1.30±0.22	<0.001***
	MB	1.53±0.14	1.14±0.26	<0.001***
	CR	1.55±0.20	1.51±0.23	0.333
	CM	1.54±0.19	1.54±0.19	0.182
Probing depth (mm)	RB	2.36±0.29	2.19±0.27	<0.001***
	MB	2.35±0.32	2.11±0.28	<0.001***
	CR	2.37±0.29	2.35±0.28	0.595
	CM	2.39±0.34	2.36±0.33	0.254
Colony-forming units ($\times 10^3$ CFU/mL)	RB	86.75±30.79	69.30±27.76	<0.001***
	MB	89.63±32.60	52.80±32.22	<0.001***
	CR	89.20±36.08	94.23±40.53	0.124
	CM	90.10±30.81	93.43±37.78	0.397

Paired t-test, P>0.05=no significant difference, **P<0.001=highly significant difference

Parameter	Group	T_0 (Mean ± SD)	T_1 (Mean ± SD)	P-value
Gingival index (GI)	RB	1.49±0.13	1.25±0.19	<0.001***
	MB	1.50±0.20	1.06±0.27	<0.001***
	CR	1.52±0.19	1.49±0.24	0.786
	CM	1.50±0.16	1.46±0.24	0.637
Bleeding on probing (BOP)	RB	1.49±0.32	1.04±0.37	<0.001***
	MB	1.49±0.35	0.74±0.45	<0.001***
	CR	1.62±0.31	1.43±0.39	0.002**
	CM	1.51±0.28	1.33±0.39	0.027*

Wilcoxon signed-rank test, P>0.05=no significant difference *P<0.05=significant difference, **P<0.01=highly significant difference.***P<0.001=very highly significant difference

RB: Riboflavin, MB: Methylene blue, CR: Riboflavin Control, CM: Methylene blue Control, T0: at base line, T1: after 28

Table 3: Intergroup comparisons of different study parameters

Parameter	Time Interval	RB (Mean ± SD)	MB (Mean ± SD)	CR (Mean ± SD)	CM (Mean ± SD)	P-value
PI	T_0	1.56±0.20	1.53±0.14	1.55±0.20	1.54±0.19	0.833
PI	T_1	1.30±0.22	1.14±0.26	1.51±0.23	1.49±0.23	<0.001***
PD(mm)	T_0	2.36±0.29	2.35±0.32	2.37±0.29	2.39±0.34	0.967
PD(mm)	T_1	2.19±0.27	2.11±0.28	2.35±0.28	2.36±0.33	<0.001***
CFU($\times 10^3$ CFU/mL)	T_0	86.75±30.79	89.63±32.60	89.20±36.08	90.10±30.81	0.969
CFU ($\times 10^3$ CFU/mL)	T_1	69.30±27.76	52.80±32.22	94.23±40.53	93.43±37.78	<0.001***
Comparison among groups at T_1						
Parameter	RB vs. MB	RB vs. CR	RB vs. CM	MB vs. CR	MB vs. CM	CM vs. CR
PI	0.022*	<0.001***	0.002**	<0.001***	<0.001***	0.976
PD (mm)	0.520	0.074	0.053	<0.001***	<0.001***	0.999
CFU ($\times 10^3$ CFU/mL)	0.017 *	0.011*	0.010**	<0.001***	<0.001***	0.961

One-way ANOVA test followed by Tukey Post hoc test, P>0.05=no significant difference, *P<0.05=significant difference, **P<0.01=highly significant difference, ***P<0.001=very highly significant difference

Parameter	Time interval	RB (Mean ± SD)	MB (Mean ± SD)	CR (Mean ± SD)	CM (Mean ± SD)	P-value
GI	T_0	1.49±0.13	1.50±0.20	1.52±0.19	1.50±0.16	0.947
GI	T_1	1.25±0.19	1.06±0.27	1.49±0.24	1.46±0.24	<0.001***
BOP	T_0	1.49±0.32	1.49±0.35	1.62±0.31	1.51±0.28	0.294
BOP	T_1	1.04±0.37	0.74±0.45	1.43±0.39	1.33±0.39	<0.001***
Comparison among groups at T_1						
Parameter	RB vs. MB	RB vs. CR	RB vs. CM	MB vs. CR	MB vs. CM	CM vs.CR
GI	0.013*	<0.001***	<0.001***	<0.001***	<0.001***	0.741
BOP	0.014*	<0.001***	0.003**	<0.001***	<0.001***	0.385

Kruskal-Wallis test followed by Mann-Whitney U test, P>0.05=no significant difference, *P<0.05=significant difference, **P<0.01=highly significant difference, ***P<0.001=very highly significant difference

RB: Riboflavin, MB: Methylene blue, CR: Riboflavin Control, CM: Methylene blue Control, T0: at base line, T1: after 28 days, PI: Plaque index, PD: Probing depth, CFU: Colony forming unit, GI: gingival index, BOP: Bleeding on probing

Table 4: Intra-examiner reliability of different study parameters

Parameter	Intraclass correlation coefficient (ICC)
Plaque index (PI)	0.93
Gingival index (GI)	0.91
Bleeding on probing (BOP)	0.96
Probing depth (PD)	0.87
Colony-forming units (CFU)	0.84

Discussion

Orthodontic treatment with fixed appliances presents significant challenges to maintaining oral hygiene, especially in younger patients (16). These appliances facilitate plaque accumulation and biofilm formation, complicating periodontal management and potentially leading to pathological inflammation and irreversible cellular changes (17,18). The gold standard for preventing such complications remains effective plaque control through proper brushing techniques and

routine prophylactic scaling (19,20).

In response to these challenges, alternative antimicrobial strategies such as PDT have been investigated (21,22). PDT uses photosensitizers that, upon activation by light, produce cytotoxic reactive species with broad-spectrum antimicrobial activity and minimal mutagenic risk (23). Moreover, since PDT exerts its effects through direct microbial cell destruction, the likelihood of developing antimicrobial resistance is considered low (24). Despite extensive research on PDT in the context of periodontal therapy, its specific application in adolescents undergoing fixed orthodontic treatment remains insufficiently explored.

Intergroup comparison at T1 showed that the plaque index decreased by 0.39 and the gingival index decreased by 0.44 in the MB group, which was the highest among others. Also, the plaque index and gingival index scores in the RB group were significantly lower than those in the CM and CR groups. This suggests that methylene blue and riboflavin were both effective in reducing plaque and gingival index scores, while methylene blue showed a significant difference (P=0.022 and P=0.013, respectively) compared to riboflavin.

The intergroup comparison at T1 revealed that the probing depth was significantly lower in the MB group than in the CM and CR groups. The probing depth in the RB, CM, and CR groups did not differ significantly. This suggests that methylene blue was more effective in decreasing probing depth, while riboflavin also showed a decrease in probing depth; however, the difference was not significant (P=0.052). These results were consistent with those of Abellan et al. (6), who showed reduced probing depth in the methylene blue photosensitizer group.

Intergroup comparisons showed that the BOP score was significantly lower in the methylene blue group than in the RB, CM, and CR groups. Additionally, BOP scores in the RB group were significantly lower than those in the CM and CR groups, indicating that both methylene blue and riboflavin were effective in reducing BOP scores. In contrast, methylene blue showed a more significant difference (P=0.014) compared to riboflavin.

Intergroup comparisons showed that colony-forming units were significantly lower in the MB group (52.80×103/mL) compared to the RB (69.30×103/mL), CM (93.43×103/mL), and CR (94.23×103/mL) groups. Furthermore, the colony-forming units in the RB group were significantly lower than those in the CM and CR groups, indicating that both methylene blue and riboflavin were effective in reducing colony-forming units. In contrast, methylene blue showed a significant difference (P=0.017) from riboflavin. Alqerban (25) studied the antibacterial properties of rose bengal and riboflavin in modified orthodontic resin for orthodontic bonding, reporting that both riboflavin and rose bengal exhibited substantial antibacterial properties. The present study demonstrated that PDT with riboflavin showed significant improvements in periodontal health parameters as well as a reduction in the bacteriological counts of oral microflora. Top of Form

In this study, both riboflavin and methylene blue PDT significantly reduced PI, GI, BOP, PD, and CFU counts in the experimental groups, consistent with previous findings. Gómez et al. (5) demonstrated that methylene blue PDT, when used as an adjunct to ultrasonic scaling, effectively reduced plaque accumulation, gingival inflammation, and probing depth. Similarly, Abellán et al. (6) reported significant improvements in these parameters in patients with fixed orthodontic appliances. Studies by Al Nazeh et al. (7) and Baeshen et al. (8) also revealed reductions in plaque scores, BOP, and microbial load following PDT.

After 28 days, comparisons between the riboflavin and methylene blue groups revealed significant differences in PI, GI, BOP, and CFU counts, suggesting that methylene blue was the most effective photosensitizer. However, no significant difference in PD was observed between the two groups. Also, there was a significant improvement in BOP in both control groups. The reduction in BOP in both control groups, despite unchanged plaque and gingival indices, likely reflects transient improvements in gingival inflammation rather than effective plaque control. This may be due to the Hawthorne effect, where study participation prompts better oral hygiene behavior, though not necessarily with proper technique or interdental cleaning. BOP is a sensitive marker of acute inflammation and may respond to minimal interventions, whereas plaque and gingival indices require sustained mechanical disruption to show measurable change. These findings underscore the importance of reinforcing comprehensive oral hygiene practices in clinical protocols.

Etemadi et al. (26) reported that riboflavin activated by blue light effectively reduced periodontopathogenic bacteria. Similarly, Moradi et al. (10) and Afrasiabi et al. (11) demonstrated its effectiveness in root canal disinfection against Enterococcus faecalis, while Kamran et al. (12) confirmed its antibacterial activity against Streptococcus mutans and Streptococcus sanguinis. Collectively, these findings highlight the potential of riboflavin-based PDT as a promising adjunct for improving periodontal health in orthodontic

patients.

Both PDT protocols significantly improved periodontal outcomes and reduced microbial load. While methylene blue demonstrated greater overall efficacy, riboflavin-based PDT remains a viable and cost-effective alternative—particularly for clinicians without access to laser equipment—as it can be administered using standard LED light-curing units commonly available in orthodontic settings. Patients reported no adverse effects; however, staining of elastomeric modules was noted. To address this aesthetic concern, the use of dark-colored elastomeric modules or stainless-steel ligature wires is recommended. While safety remains a fundamental consideration, riboflavin's extensive pharmacological and nutritional use—without significant adverse events at conventional oral or systemic doses—attests to its excellent tolerability.

Methylene blue and riboflavin are both effective photosensitizing agents in dental photodynamic therapy, each offering distinct therapeutic benefits. Methylene blue, activated by red light, enables deeper tissue penetration and generates a higher yield of singlet oxygen, making it particularly effective in managing periodontal infections and endodontic biofilms. Riboflavin, activated by blue light, is more suitable for superficial applications such as enamel remineralization, early carious lesion control, and adjunctive improvement of oral hygiene through plaque reduction. While methylene blue provides superior antimicrobial efficacy and photostability, riboflavin supports minimally invasive, surface-level interventions that contribute to preventive dental care and enhanced patient compliance.

Fiegler-Rudol et al. (9) highlighted the potential of riboflavin-mediated photodynamic therapy (PDT) as a promising adjunct in periodontal care, demonstrating enhanced antimicrobial efficacy and favorable clinical outcomes. Despite variability in study designs and outcome measures, which limit definitive conclusions, the aggregated evidence suggests that riboflavin-mediated PDT performs comparably—and in some cases superiorly—to conventional modalities such as mechanical debridement. Noteworthy benefits include a significant reduction of biofilm, minimal tooth staining, and a lower likelihood of contributing to antibiotic resistance.

Łopaciński et al. (27) suggested riboflavin-mediated antimicrobial photodynamic therapy (PDT) as a promising, non-invasive option for managing Candida infections, including those resistant to fluconazole. Upon activation with blue light, riboflavin generates reactive oxygen species that markedly reduce fungal viability and biofilm mass while sparing host tissues. The compiled data show that riboflavin PDT can achieve antifungal effects comparable to those of conventional agents, yet with the added benefits of localized action and minimal systemic toxicity. Moreover, preliminary evidence suggests that combining riboflavin PDT with adjunctive therapies may further amplify its efficacy, supporting its potential role as both an alternative and complement to standard antifungal treatment.

In this preliminary RCT, a formal cost analysis was not conducted; nonetheless, both riboflavin and methylene blue are inexpensive photosensitizers. Riboflavin-based PDT can be administered with a low-cost LED array and requires no disposables. In contrast, methylene blue-based PDT depends on a more expensive laser system and single-use fiber-optic tips for each session. This equipment-related cost disparity further underscores the practical advantage of riboflavin-based PDT, particularly in settings with constrained resources.

In our study, riboflavin PDT was administered weekly over four weeks, independent of routine orthodontic appointments, which typically occur every 4–6 weeks. This intensified protocol allowed for targeted microbial control and periodontal support. Integrating such adjunctive care between standard visits may offer enhanced oral health benefits without disrupting the conventional orthodontic workflow.

Limitations

This study had several limitations. First, the follow-up period was relatively short, which restricts our ability to determine the long-term stability of periodontal improvements after photodynamic therapy. Longer observation periods are necessary to assess whether the microbial and clinical changes induced by PDT persist over time.

Second, although a split-mouth randomized design minimizes inter-patient variability, it may introduce a potential carry-over or cross-contamination effect between quadrants, particularly in microbiological outcomes, even though efforts were made to maintain strict isolation and prevent cross-site influence.

Third, the study was single-masked; the operator delivering the interventions was not blinded, which could introduce performance bias. Complete blinding was not feasible due to the distinct characteristics of the photosensitizers, but this remains a limitation.

Fourth, microbial analysis was performed using

culture-based methods and focused primarily on total anaerobic counts. This approach does not allow species-level identification or assessment of complex microbial shifts.

Finally, inflammatory biomarkers (such as IL-1β, TNF-α, or MMP-8) were not assessed. Including biochemical markers could strengthen the interpretation of periodontal improvement beyond clinical parameters alone.

Conclusion

Both riboflavin and methylene blue PDT were associated with reductions in plaque index, gingival index, bleeding on probing, probing depth, and microbial colony-forming units in patients undergoing fixed orthodontic treatment. Although methylene blue PDT showed comparatively greater improvements in some periodontal parameters, riboflavin PDT may still serve as a feasible and accessible option, particularly in settings where laser-based systems are not available and LED light-curing units are used instead.

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None.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Data Availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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