Effect of Nd:YAG Laser-Assisted Non-Surgical Periodontal Therapy on Clinical Periodontal and Serum Biomarkers in Patients With and Without Coronary Artery Disease: A Short-Term Pilot Study

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Background/Objective: We hypothesized that nonsurgical-periodontal-therapy (NSPT) with adjunct Nd:YAG laser therapy is more effective in reducing periodontal inflammatory parameters (plaque index [PI], bleeding-on-probing [BOP], and probing-pocket-depth [PPD]) and serum interleukin-1beta (IL-1β) and matrix metalloproteinase-9 (MMP-9) levels in patients with and without coronary artery disease (CAD) than NSPT alone. The aim of this short-term pilot study was to assess the effect of NSPT + Nd:YAG laser therapy on periodontal parameters and serum IL-1β and MMP-9 levels in patients with and without CAD.

Study Design: A prospective randomized clinical study was conducted on 87 patients who were divided into two groups: Group-1: 44 patients with CAD and periodontal disease (PD) and Group-2: 43 patients with PD alone. Treatment-wise, these individuals were randomly divided into two subgroups: (i) NSPT alone and (ii) NSPT + Nd:YAG laser therapy. Demographic information was collected using a self-completed questionnaire. Periodontal parameters (PI, BOP, and PPD) and serum IL-1β and MMP-9 levels were measured at baseline and after 3 months of treatment. *P*-values <0.05 were considered statistically significant.

Results: At 3 months follow-up, PI ($P < 0.01$), BOP ($P < 0.01$), PPD ≥ 4 mm ($P < 0.01$), and serum IL-1β ($P < 0.01$) and MMP-9 ($P < 0.01$) levels were significantly higher in patients treated with NSPT alone than those treated with NSPT + Nd:YAG laser therapy. Among patients that underwent NSPT + laser therapy in both groups, periodontal parameters and serum IL-1β, and MMP-9 levels were comparable at 3-months follow-up.

Conclusion: NSPT + Nd:YAG laser therapy may be more effective in reducing periodontal inflammation and serum IL-1β and MMP-9 levels in patients with and without CAD than NSPT alone. Lasers Surg. Med.

Key words: coronary artery disease; laser; non-surgical periodontal therapy; periodontal inflammation

INTRODUCTION

Coronary artery disease (CAD) or coronary heart disease is the leading cause of mortality among males and females in the United States [1]. It is a chronic inflammatory disease of the heart that is characterized by atherosclerosis in the epicardial coronary arteries [2–4]. The key factor associated with the development and progressions of the atherosclerotic plaque are the monocyte-macrophage lineage cells. Activated macrophages secrete pro-inflammatory cytokines, such as interleukin 1-beta (IL-1β) and matrix metalloproteinase-9 (MMP-9) that may influence the mechanisms involved in vascular occlusion and repair [5,6]. It has been reported that serum concentrations of IL-1β and MMP-9 are elevated in patients with CAD compared with individuals without cardiovascular disorders (CVD) [6].

Periodontal disease (PD) is a bacterially-induced inflammatory condition that affects the supporting structures of teeth (gingiva, periodontal ligament, and alveolar bone) [7]. The most common cause of PD is poor oral hygiene maintenance [8,9]; however, in a systematic review, the reported relative risk for CAD was significantly

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higher in patients with PD than patients without PD [10]. Moreover, raised levels of proinflammatory cytokines including IL-1β and MMP-9 have been identified in the serum and gingival crevicular fluid samples collected from patients with PD [11,12]. In patients with PD, excessive production of IL-1β and MMP-9 has been associated with the degradation of the extracellular matrix and increased osteoclastic activity that leads to alveolar bone resorption [13,14].

Non-surgical periodontal therapy (NSPT) using ultrasonic scalers and/or hand instruments (such as curettes) is usually performed for the management of PD [15–17]; however, NSPT alone is often ineffective in completely eliminating pathogenic microbes and their products from periodontal pockets [18,19]. Preliminary studies [18,20–22] have suggested that NSPT with adjunct neodymium-doped:yttrium, aluminum, and garnet (Nd:YAG) laser therapy may be more effective in reducing periodontal inflammation than when NSPT is used alone. Specifically, results from a recent short-term clinical study reported that NSPT with adjunct Nd:YAG laser therapy is more effective than NSPT alone in the treatment of PD in systemically healthy individuals as well as in immunocompromised patients [20]. The Nd:YAG laser has been used for many years in soft tissue and periodontal surgery [23,24]. It is hypothesized that NSPT with adjunct Nd:YAG laser therapy is more effective in reducing periodontal inflammatory parameters (plaque index [PI], bleeding on probing [BOP], and probing pocket depth [PPD]) and serum IL-1β and MMP-9 levels in patients with and without CAD as compared to NSPT alone.

The aim of the present short-term pilot study was to assess the effect of NSPT with adjunct Nd:YAG laser therapy on PI, BOP, and PPD and serum IL-1β and MMP-9 levels in patients with and without CAD.

METHODS

Ethical Approval and Consent

The study was approved by the Research Ethics Review Committee of the College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia. A consent form was presented to all volunteering individuals. It was mandatory for all individuals to have read and signed the consent form before being included in the present study.

Eligibility Criteria

The recommendation of the Consolidated Standards of Reporting Trials (CONSORT) statement were followed [25]. The inclusion criteria were as follows: (i) patients with medically diagnosed CAD (that is, patients with a stenosis of >50% diameter in at least one of the major coronary artery segments) [26]; (ii) self-reported systemically healthy individuals; (iii) and patients with moderate PD (PPD $\geq$ 4 mm in at least 30% sites). The following exclusion criteria were imposed: (i) Edentulous patients; (ii) tobacco smokers and smokeless tobacco chewers; (iii) patients with misaligned teeth; (iv) patients with systemic disorders other than CAD (such as diabetes mellitus, acquired immune deficiency syndrome/HIV, hepatitis, prediabetes, and renal disorders); (v) third molars; and (vi) patients who reported to have used antibiotics, non-steroidal anti-inflammatory drugs and/or steroids within the past 3 months.

Recruitment of Participants

An information sheet, that described the purpose of the study and design was provided to individuals visiting the cardiovascular and the oral healthcare units of a local hospital in Riyadh, Saudi Arabia. All volunteering individuals ($n = 87$) signed a consent form and were invited to the oral healthcare unit for further investigations.

Study Participants

Participants ($n = 87$) were divided into two groups as follows: (i) CAD + PD: 44 patients; and (ii) PD alone: 43 patients. Individuals in each group were randomly assigned to receive either of the following periodontal therapies: (i) NSPT alone or (ii) NSPT with adjunct Nd:YAG laser therapy. Randomization was done by tossing a coin. In patients with CAD + PD, 22 patients received NSPT alone and 22 patients received NSPT with adjunct Nd:YAG laser therapy; whereas in patients with PD alone, 22 patients received NSPT alone and 21 patients received NSPT with adjunct Nd:YAG laser therapy (Fig. 1).

Questionnaire

A self-completed questionnaire was presented to all participants. The questionnaire gathered information regarding the age, gender, duration of CAD, family history of CVD, family history of PD, and daily oral hygiene maintenance protocols (tooth brushing once or more daily and flossing once or more daily).

Clinical Periodontal Parameters

Full mouth PI [27], BOP [27], and PPD [27] were measured at six sites per tooth (mesiobuccal, midbuccal,

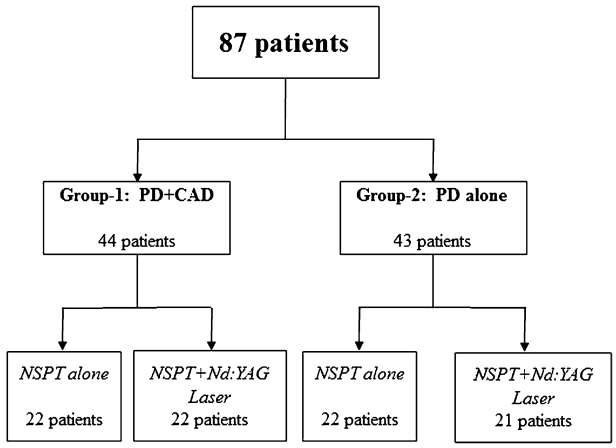

Fig. 1. Study participants and grouping.

distobuccal, distolingual/distopalatal, midlingual/midpalatal, and mesiolingual/mesiopalatal) on all maxillary and mandibular teeth. One well-trained investigator blinded to the study groups performed the clinical periodontal examination. The overall *kappa* value for intra-examiner reliability was 0.88. PPD was measured to the nearest millimetre with a graded probe (Hu Friedy, IL, Chicago) [27,28]. Fractured teeth with embedded root remnants were also excluded. Periodontal parameters were measured at baseline and after 3 months of NSPT with and without adjunct Nd:YAG laser therapy.

Laser Parameters

The Nd:YAG laser system (Genius Dental, Tureby, Denmark) was used that emits pulsed light at 1,064 nanometers. To avoid thermal effect and maintain the optimal therapeutic effect, the instrument was set at level five using the following parameters: average output: 4 watt (W); energy per pulse: 80 millijoules; pulse width: 350 milliseconds, pulse-repetition rate: 50 hertz; pulse peak power: 12 watt; average power density at the fiber end: 1,430 watts per square centimeters (W/cm^2); and peak power density: 85,800 W/cm^2 [18]. Laser treatment was accompanied by air and water-cooling. Irradiation parameters were governed through the fiber diameter, treatment time, power of the laser at the tip of the fiber, and surface area of the irradiation site. The fiber diameter was 600 microns. Water-cooling and air-cooling were always used during irradiation. The time spent on each tooth varied between 60 and 120 seconds, depending on accessibility. The laser energy per treated site was 240–480 J. The power density and peak-power density were calculated by a hypothetical 100% emission through the small fiber tip. However, the energy was not emitted solely from the tip of the fiber; there was also considerable lateral emission. Because of the high uncertainty about the total area of irradiated tissue, the energy density (joules per square centimeter) was not calculated.

The laser fiber was placed in the periodontal pocket almost parallel to the tooth and moving from distal to mesial directions continuously on the buccal and the lingual aspect of the tooth. The fiber was held in a constant motion in contact with the pocket epithelial lining almost parallel to the long axis of the root.

Blood Samples and Measurement of Serum IL-1β and MMP-9 Levels

From all participants, peripheral venous blood samples were collected in test tubes and allowed to clot for 30 minutes at room temperature. The test tubes were then centrifuged at 3,000*g* for 15 minutes at 4°C. Serum samples were collected and stored at −80°C until further investigation. All serum samples were assessed within 7 days of sampling. Serum levels of IL-1β (Milliplex®, Billerica, MA) and MMP-9 (Amersham Pharmacia Biotech Inc., Piscataway, NJ) were measured using commercially available enzyme linked immunosorbent assay (ELISA, Molecular Devices, Vmax, Sunnyvale, CA) kits [29]. Fifty microliters of serum was used in each analysis. In summary, for determination of serum IL-1β and MMP-9 levels, 96-well microplates were coated with antibodies against IL-1β and MMP-9 in separate plates in duplicates. Diluted samples of IL-1β and MMP-9 and standards (provided with the respective kits) to the 96-well microplate and incubated overnight at 4°C. The wells were washed four times in a washer (BioTek™ 405™ Touch Microplate Washer, VT) and secondary antibodies provided by the manufacturer were added to the respective plates. p-aminophenylmercuric acetate was added and the plates were incubated for 2 hours at 37°C. The modified prodetection reagent (in 50 mm Tris HCL pH 7.6) was added to each well and the resultant color was read in a microtitre plate spectrophotometer (BioTek, VT) at 405 nanometers. A standard curve was generated by plotting the $absorbance_{405}$ against the standard of IL-1β (ng/L) and MMP-9 (ng/L) and the concentrations were determined by interpolating their $absorbance_{405}$ values using a standard curve [30,31]. Serum IL-1β and MMP-9 levels were measured at baseline and after 3 months of NSPT with and without adjunct Nd:YAG laser therapy.

Statistical Analysis

Data were statistically assessed using the SPSS software (SPS Version 18.0, IL). Values were presented as means ± 95% confidence intervals and comparisons were made using one-way analysis of variance. For multiple comparisons, the Bonferroni *post hoc* test was used. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Characteristics of the Study Population

All individuals that volunteered to participate in the present study were male. In patients with CAD + PD ($n = 44$), the mean age of patients that received NSPT alone ($n = 22$), and NSPT + laser ($n = 22$) was 52.4 ± 2.7 years and 58.2 ± 3.1 years, respectively. In patients with PD alone ($n = 43$), the mean age of patients that received NSPT alone ($n = 22$) and NSPT + laser ($n = 21$) was 55.7 ± 2.2 years and 60.1 ± 1.8 years, respectively. Among patients with CAD + PD, a family history of CVD was reported by 59.1% individuals that received NSPT alone and 63.6% individuals that received NSPT + laser therapy. In patients with PD alone, a family history of CVD was reported by 45.5% individuals that received NSPT alone and 47.6% individuals that received NSPT + laser therapy. In CAD + PD group, 27.2% (NSPT alone) and 18.2% (NSPT + laser) reported to have a family history of PD. In patients with PD alone, a family history of PD was reported by 22.7% individuals that received NSPT alone and 23.8% individuals that received NSPT + laser therapy (Table 1).

In patients with CAD + PD, tooth brushing once daily was reported by 90.9% patients that received NSPT alone and 86.4% individuals that received NSPT + laser therapy. In patients with PD alone, 86.3% individuals that received NSPT alone and 76.2% individuals that received

TABLE 1. General Characteristics of the Study Population

	Patients with CAD + PD		Patients with PD alone	
	NSPT alone	NSPT + laser	NSPT alone	NSPT + laser
n	22	22	22	21
Gender (male:female)	22:0	22:0	22:0	21:0
Age in years (Mean ± CI)	52.4 ± 2.7	58.2 ± 3.1	55.7 ± 2.2	60.1 ± 1.8
Duration of CAD in years (Mean ± CI)	5.1 ± 1.5	5.8 ± 1.3	–	–
Family history of cardiovascular disease (%)				
Yes	59.1%	63.6%	45.5%	47.6
No	18.1%	22.7%	9.1%	9.5%
I do not know	22.8%	13.7%	45.4%	42.9%
Family history of periodontal disease (%)				
Yes	27.2%	18.2%	22.7%	23.8%
No	–	–	18.1%	19%
I do not know	72.8%	81.8%	59.2%	57.2%
Daily tooth brushing (%)				
Once	90.9%	86.4%	86.3%	76.2%
Twice	9.1%	13.6	13.7%	23.8%
Three times or more	–	–	–	–
Daily dental flossing (%)				
Once	–	–	–	–
Twice	–	–	–	–
Three times or more	–	–	–	–

NSPT + laser therapy reported to brush their teeth once daily. None of the individuals in either group reported to have ever used dental floss (Table 1). All patients with CAD had been prescribed anticoagulants, beta-blockers and statins by their healthcare providers.

Clinical Periodontal Parameters: NSPT Alone Compared With NSPT + Laser

CAD + PD group. At 3 months follow-up, PI ($P < 0.01$), BOP ($P < 0.01$), and PPD ≥ 4 mm ($P < 0.01$) were significantly higher in patients treated with NSPT alone compared with those treated with NSPT with adjunct Nd:YAG laser therapy (Table 2).

PD alone group. At 3 months follow-up, PI ($P < 0.01$), BOP ($P < 0.01$) and PPD ≥ 4 mm ($P < 0.01$) were significantly higher in patients treated with NSPT alone compared with those treated with NSPT with adjunct Nd:YAG laser therapy (Table 2).

CAD + PD versus PD alone groups. After 3-months of follow-up, there was no statistically significant difference in PI, BOP, and PPD ≥ 4 mm in NSPT alone and NSPT + laser subgroups among patients with CAD + PD and PD alone (Table 2).

Serum Interleukin 1-Beta and Matrix Metalloproteinase-9 Levels:: NSPT Alone Compared With NSPT + Laser

CAD + PD group. At 3-months follow-up, individuals who had received NSPT + laser therapy demonstrated significantly lower serum IL-1β ($P < 0.05$) and MMP-9 ($P < 0.05$) levels as compared to patients who had undergone NSPT alone (Table 3).

TABLE 2. Periodontal Parameters at Baseline and After 3-Months of Periodontal Therapy in Patients With CAD + PD and PD Alone

	Patients with CAD + PD				Patients with PD alone			
	Baseline		3-months follow-up		Baseline		3-months follow-up	
	NSPT alone	NSPT + laser	NSPT alone	NSPT + laser	NSPT alone	NSPT + laser	NSPT alone	NSPT + laser
PI	68.5 ± 2.56	73.3 ± 1.91	21.5 ± 1.34[a]	8.8 ± 0.13	48.5 ± 1.34	51.2 ± 1.53	25.5 ± 1.72[b]	7.6 ± 0.32
BOP	75.2 ± 2.23	72.3 ± 3.26	23.4 ± 2[a]	4.2 ± 0.27	59.1 ± 1.39	58.4 ± 1.7	25.7 ± 0.74[b]	3.3 ± 0.17
PPD ≥ 4 mm	48.7 ± 1.18	52.4 ± 2.4	18.9 ± 0.53[a]	2.2 ± 0.08	36.5 ± 1.77	39.7 ± 0.97	14.7 ± 0.86[b]	1.9 ± 0.11

[a]Compared with NSPT + laser at 3-months follow-up in patients with CAD + PD ($P < 0.01$).
[b]Compared with NSPT + Laser at 3-months follow-up in patients with PD alone ($P < 0.01$).

PD alone group. At 3-months follow-up, individuals who had received NSPT + laser therapy demonstrated significantly lower serum IL-1β ($P < 0.05$) and MMP-9 ($P < 0.05$) levels as compared to patients who had undergone NSPT alone (Table 3).

CAD + PD versus PD alone groups. At 3-months follow-up, among patients that received NSPT alone, serum IL-1β ($P < 0.05$) and MMP-9 ($P < 0.05$) levels were higher in patients with CAD + PD compared with patients with PD alone. Among PD patients with and without CAD, serum IL-1β, and MMP-9 levels were comparable at 3-months follow-up in patients that underwent NSPT + laser therapy (Table 3).

DISCUSSION

To our knowledge from indexed literature, this is the first study that has investigated the effect of NSPT with and without adjunct Nd:YAG laser therapy on the clinical and immunologic biomarkers of inflammation in patients with PD with and without CAD. In the present study, it was hypothesized that NSPT with adjunct Nd:YAG laser therapy is more effective in reducing the clinical markers of periodontal inflammation (PI, BOP, and PPD) and serum IL-1β and MMP-9 levels in patients with and without CAD as compared to NSPT alone. The present results support this hypothesis since there was a significant reduction in clinical periodontal (PI, BOP, and PPD) and serum immunologic inflammatory biomarkers (IL-1β and MMP-9) in both groups that received NSPT + laser therapy as compared to NSPT alone (Tables 2 and 3). An explanation in this regard is that the Nd:YAG laser wavelength recommended for periodontal therapy (1064 nm) is not readily absorbed by dental hard tissues, such as cementum and/or dentin and affects only the soft tissues such as the pocket epithelial lining thereby enhancing new supportive tissue formation [32]. Moreover, it has also been reported that Nd:YAG laser therapy induces the proliferation of gingival fibroblasts, gingival epithelial cells, periodontal ligament cells, and bone mesenchymal stem cells [33–36]. This suggests that concurrent use of NSPT and Nd:YAG laser facilitates periodontal wound healing to a significantly greater extent as compared to NSPT alone in patients with and without CAD. This laser wavelength was used relatively early in the field of periodontics to reduce PPD [23,24].

An interesting finding of the present study was that although baseline clinical periodontal parameters were comparable among patients with CAD + PD and patients with PD alone, serum IL-1β, and MMP-9 levels were significantly higher among patients with CAD + PD compared with patients with PD alone. One explanation for this outcome is that T-cell activation in patients with CAD secretes interferon gamma, which decreases collagen production in smooth muscles; whereas activated macrophages secrete matrix metalloproteinases that causes proteolytic degradation of collagen thereby making the fibrous cap weak and prone to rupture [37]. This adhesive interaction between vascular cells play a role in causing the inflammatory response [37]. Moreover, high levels of proinflammatory cytokines have also been identified in the periodontal tissues and gingival crevicular fluid of patients with PD [12]. These results suggest that the systemic burden of inflammation is significantly higher among patients with CAD and PD compared with patients with PD alone.

The present results support previous studies, which have shown that a family history of CVD is a classical risk-factor for CAD [38,39]. In the present study, approximately 61% with CAD had a family history of CVD. However, among patients with PD alone, nearly 50% patients with PD had a history of CVD. It is therefore hypothesized that nearly half of these patients are at risk of developing CVD and that there is a likelihood that some of these individuals

TABLE 3. Serum IL-1β and MMP-9 Levels at Baseline and After 3-Months of Periodontal Therapy in Patients With CAD + PD and PD Alone

	Patients with CAD + PD				Patients with PD alone			
	Baseline		3-months follow-up		Baseline		3-months follow-up	
Serum Cytokines	NSPT alone	NSPT + laser	NSPT alone	NSPT + laser	NSPT alone	NSPT + laser	NSPT alone	NSPT + laser
IL-1β (ng/L)	67.5 ± 2.6	70.7 ± 1.2	32.4 ± 0.4[a,b]	4.5 ± 0.4	30.5 ± 3.1	27.4 ± 1.6	13.5 ± 9.6[e]	3.7 ± 0.5
MMP-9 (ng/L)	3871.5 ± 25	4005.6 ± 49	2158.7 ± 18[c,d]	426.2 ± 15	1627.7 ± 87	1854.3 ± 80	932.4 ± 21[f]	401.8 ± 21

[a]Compared with NSPT + laser at 3-months follow-up in patients with CAD + PD ($P < 0.05$).
[b]Compared with NSPT alone at 3-months follow-up in patients with PD alone ($P < 0.01$).
[c]Compared with NSPT + laser at 3-months follow-up in patients with CAD + PD ($P < 0.001$).
[d]Compared with NSPT alone at 3-months follow-up in patients with PD alone ($P < 0.01$).
[e]Compared with NSPT + laser at 3-months follow-up in patients with PD alone ($P < 0.05$).
[f]Compared with NSPT + laser at 3-months follow-up in patients with PD alone ($P < 0.001$).

may even be undiagnosed. Studies [40,41] have also reported that PD is a risk factor for CVD. In the present study, nearly 70% PD individuals (with and without CAD) were unaware whether they had a family history of PD or not. It is therefore emphasized that patient education (in terms of systemic as well as oral hygiene maintenance) should be considered as an integral component in the overall treatment strategy for the management of patients with CVD. Likewise, presence of PD in undiagnosed elderly patients (60 years and older) may also be an indicator for CVD in undiagnosed individuals.

It has been reported that statins reduce the secretion of MMP-9 by smooth muscle cells and macrophages [42,43]. A surprising outcome of the present study was that baseline MMP-9 levels were higher in patients with CAD although they had been prescribed statins by their healthcare providers. It is possible that the patients were not adhering to the prescribed medications including statins. A number of factors that may have influenced the frequency of medication consumption include an underprivileged socioeconomic status (SES) and poor education of the patient [44]. According to Alsabbagh et al. [45], an underprivileged SES is a critical factor associated with an individuals' non-adherence to medications prescribed by healthcare providers. Lamentably, SES and education status of the participants remained uninvestigated in the present study; however, their influence on the clinical periodontal parameters and serum cytokine profiles in PD with and without CAD cannot be overlooked. Taking into consideration the public health significance of this outcome, failure in taking SES into account may thwart targeting of interventions for those in need.

It is important to mention that at the initiation of the present research study, an information sheet, that described the purpose of the study, and design was provided to volunteering individuals. There is a possibility that the volunteering individuals became more cautious of their oral hygiene status and modified their oral hygiene regimes by brushing or flossing their teeth more often, which may have biased the present results. Moreover, it may also be debated upon that beneficial effect of NSPT + laser therapy may last for a limited duration in individuals who fail to regularly maintain their oral hygiene status after therapy. In this regard, a limitation of the present study is that the oral hygiene maintenance survey was performed at the beginning of the trial. There might have been a difference in the participants' daily oral hygiene maintenance protocols at the end of the study period; however, this aspect remained unaddressed in the present investigation. It is pertinent to mention that patients with self-reported systemic diseases in addition to CAD (such as poorly-controlled diabetes mellitus and renal disorders) were excluded in the present study. It is well-known that serum levels of proinflammatory cytokines are significantly higher in immunocompromised patients compared with systemically healthy individuals [27,46]. It is therefore speculated that the beneficial effects of NSPT + Nd:YAG laser therapy in the treatment of periodontal inflammation are compromised in diabetic patients with CAD as compared to patients with CAD alone. Furthermore, tobacco smokers were excluded in the present study. Since habitual tobacco smoking can compromise the outcomes of periodontal therapy [47]; it is probable that the outcomes of NSPT (with or without adjunct Nd:YAG therapy are compromized in PD patients with and without CAD. Further long-term clinical trials are needed in this regard.

CONCLUSION

Within the limits of the present study, it is concluded that Nd:YAG laser-assisted NSPT therapy may be more effective in reducing periodontal inflammatory parameters and serum IL-1β and MMP-9 levels in patients with and without CAD.

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