ORIGINAL STUDY

The Effect of Low-level Laser Therapy as an Adjunct to Non-surgical Therapy on Inducible Nitric Oxide Synthase Salivary Levels in Different Age Groups of Patients: A Randomized Clinical Trial

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Abstract

Purpose: The present clinical investigation aimed to evaluate the effect of low-level laser treatment on the clinical parameters and checking levels of inducible nitric oxide synthase (iNOS) in individuals with stage II periodontitis across various age groups. Patient and methods: The current investigation has been carried out on 44 locations in 44 patients. Randomly selected locations were chosen from each studied case, and divided into four groups: group A consisted of 11 sites in elderly patients who underwent scaling combined with root planning exclusively. Group B included 11 sites in young patients who also got scaling and root planning exclusively, group C 11 sites in old age who received diode laser therapy, and group D: 11 sites in young age group received low-level laser (diode laser). The clinical examination used the following clinical parameters: clinical attachment level, plaque index, and Gingival index. An Enzyme-Linked Immunosorbent Assay study was conducted on iNOS. Results: Baseline and 3-month postoperative groups were compared and showed significant differences in iNOS levels which was influenced by the treatment in the young age group (nonsurgical and laser), but showed no significant difference in the old age group on both types of treatment (nonsurgical and laser). Conclusion: This clinical investigation showed that whereas non-surgical techniques in conjunction with low-level laser treatment were unsuccessful in the elderly population, it was helpful in the young group with stage II periodontitis.

Keywords: Diode laser, Inducible nitric oxide synthase, Inflammageing, Old age, Periodontal disease

1. Introduction

Periodontal diseases are pathological illnesses that affect the tissues supporting the teeth. These problems are caused by the development of bacterial biofilms, which trigger a reaction from the host's immune system. After the connective tissue above the gumline is destroyed, further loss of periodontal ligament fibers can lead to a loss of support for the bone that holds the teeth in place. Clinically, this may be seen as a loss of attachment between the gum and tooth at the cemento-enamel junction and can cause inflammation in the gum that is noticeable through redness, swelling, and bleeding when probed. Other signs of this condition include the formation of pockets around the teeth, receding gingiva, involvement of furcation areas, and eventually, loss of bone around the teeth seen on dental radiography [1e3].

Based on histological or clinical signs of inflammatory processes, and modifications to the colour and form of the gingiva with bleeding on probing, Page and Schroeder divided periodontal disorders into four stages in the year 1976. From the initial

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lesion on a spectrum to the advanced lesion, marked by attachment loss, the stages were as follows. Periodontitis had been classified based on the disease's progression throughout the 1989 World Workshop in Clinical Periodontics [4].

In 2017, a novel categorization method was created using up-to-date information. The method is multifaceted and includes a stage and grading system. The degree of loss of attachment and radiographic bone loss indicate the complexity and severity of the illness, which in turn determines the staging approach that characterizes the breadth and spread of periodontitis. The grading method assesses the speed at which periodontitis advances and may categorize people based on their likelihood of disease development, including variables like overall health and smoking habits. Utilizing preexisting datasets is essential for the implementation and verification of the novel categorization system [3,4].

Ageing is a biological phenomenon characterized by a progressive deterioration in physical functioning and elevated susceptibility to chronic ailments, comprising cardiovascular illnesses, cancer, and neurological disorders. Nine hallmarks associated with the ageing process have been identified through research. Some of these issues include shortened telomeres, genomic instability, epigenetic alterations, dysfunctional mitochondria, disturbed nutrition sensing, depleted stem cells, cellular senescence, with changed cellular communication. All these modifications are associated with long-lasting systemic inflammation, and these pathways have a role in the ageing process, although the exact time has not been definitively established [5].

Inflammaging is caused by a decrease in the capacity to handle different kinds of stresses, along with a progressive increase in the pro-inflammatory state. Inflammaging is mostly a result of diminished stress response capabilities in the body, along with an elevation in pro-inflammatory state. Prolonged exposure to antigenic and stress stimuli triggers a substantial portion of the ageing process. Furthermore, inflammaging is thought to be a result of immune system dysregulation that happens with ageing. This imbalance may impact the functioning immune system. Nevertheless, the intrinsic immune system tends to maintain a comparatively elevated degree of efficacy in older people in comparison to the acquired immune system. Therefore, inflammaging is closely associated with a phenomenon known as 'immunosenescence' [6].

Immunosenescence is a term used to describe the alterations that take place in the immune system of older humans. These changes can lead to negative health outcomes as the immune system becomes less able to respond to new pathogens. Essentially, immunosenescence refers to a decline in immune function due to aging. This condition also involves changes in lymphatic tissues which can lead to an increased risk of autoimmune diseases, infections, malignant tumors, and neurodegenerative disorders in older adults [7].

Nitric oxide (NO) is a free radical molecule produced from L-arginine via the activity of the Nitric oxide synthase (NOS) enzyme. INOS, also known as inducible nitric oxide synthase, is a distinct kind of NOS that has the highest capacity to produce NO. Although iNOS-mediated production of NO is advantageous in eradicating pathogens during the first stage of inflammation, it might potentially contribute to the degradation of bone in more severe forms of inflammatory diseases. Studies have demonstrated that the bacteria *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, that have been linked to periodontal disease, may stimulate the production of iNOS. This leads to increased amounts of NOS and contributes to the progression of periodontitis [8].

Treatment for periodontal disease primarily focuses on reducing bacteria by nonsurgical and surgical means to halt the progression of the inflammatory disease process. Scaling and root planning have shown significant efficacy in significantly lowering the abundance of microorganisms located below the gum line, resulting in a transformation of the subgingival biofilm's composition from anaerobic gram-negative bacteria to facultative gram-positive bacteria. However, the intended outcomes may not always be attained owing to places that are not easily reachable for the use of instruments, the ability of microbes to cause disease, the resistance of microorganisms to treatment, or even due to a weakened host [9].

Adjunctive radiation therapy has been proposed as an innovative approach for the treatment of periodontal disease. The laser has thermal and photo-disruptive effects that completely remove periodontal pathogens. Because the laser can penetrate deeper into tissues, it has excellent bactericidal and detoxification effects, making it a promising tool for nonsurgical periodontal treatment [9].

The diode laser is designed to target specific substances such as melanin, hemoglobin, and pigments found in harmful bacteria, which leads to their destruction. It focuses on the unhealthy tissues while leaving the healthy ones unharmed. When inserted into periodontal pockets, the laser tip removes the soft tissue walls' epithelial lining, inflammatory

infiltrates, and scatters low-dose radiation into surrounding tissues. This process accelerates the healing process by inducing photobiomodulation, a photochemical reaction that causes cells to repair and heal wounds. This reaction increases circulation, reduces edema, and minimizes pain [9].

The objective of this research is to evaluate the effect of low-level laser therapy, when used in conjunction with non-surgical treatment, on the levels of iNOS in the saliva of chronic periodontitis studied cases across various age groups. This will be assessed by measuring salivary iNOS as a parameter of oxi-inflammaging and immunosenescence occurs in periodontal sites.

2. Patients and methods

A controlled clinical trial was done on a sample of 44 participants who had been diagnosed with periodontitis. The patients contained in this research were chosen in sequential order from those who were referred to the Department of Oral Medicine, Periodontology, Diagnosis, and Radiology at the Faculty of Dentistry for girls at Al-Azhar University. The research aimed to evaluate the efficacy of periodontal therapy.

2.1. Ethical consideration

This study has been approved by the study Ethical Committee (REC) of the Faculty of Dental Medicine for girls at Al-Azhar University.

(a) Code of approval (REC-ME-24-01).
(b) Clinical Trial.gov ID (NCT06140394).

Patients who have been diagnosed with stage II, grade (A) periodontitis and are between the ages of 40 and 60. Every patient provided their signature on an informed consent document.

The inclusion criteria were individuals without any systemic disorders, diagnosed with stage II Periodontitis, exhibiting Clinical Attachment loss ranging from 3 to 4 mm, Radiographic bone loss among 15 % and thirty three percent, absence of tooth loss caused by periodontitis, and a maximum probing depth of less than 5 mm, mostly characterized by horizontal bone loss.

The exclusion criteria included smokers and pregnant (women cases), History of antibiotics in previous three months, Treatment with any systemic drug, and studied cases who have had periodontal therapy during the last 6 months before any operation.

The studied cases had been randomly allocated into four groups:

Group A (11 patients): the first subgroup is the control group (old age) which received only nonsurgical periodontal therapy.
Group B (11 patients): the second subgroup is the control group (young age) which received only nonsurgical periodontal therapy.
Group C (11 patients): the third subgroup is the study subgroup (old age) which received nonsurgical periodontal therapy with the application of low-level laser therapy.
Group D (11 patients): the fourth subgroup is the study subgroup (young age) which received nonsurgical periodontal therapy with the application of low-level laser therapy. Study procedures:

2.2. 1-Periodontal therapy

Patients underwent root planing, scaling (both above and below the gum line), and teaching on proper dental care as part of phase one periodontal therapy. Ultrasonic and manual methods were also used to complete these processes. Reassessments of the clinical parameters were conducted at four intervals after the surgery: baseline, 4 weeks following the procedure, 3 months, and a total of 6 months. Clinical Adhesion Level, Gingival Index, Plaque Index, in addition enzyme-linked immunosorbent assay quantification for iNOS were the clinical criteria that were looked at.

2.3. Plaque index (PI)

0 ¼ There is no plaque present in the gingival region.
1 ¼ The naked eye is unable to see a film of plaque that is attached to the free gingival edge. However, the presence of this plaque may be identified by using a disclosing agent.
2 ¼ There is a buildup of soft deposits in the gingival pocket and on the gingival border of the tooth surface. These deposits are visible but do not cover more than half of the tooth.
3 ¼ An excessive amount of soft matter is present in the pocket, gingival edge, and tooth surface, covering more than half of the tooth.

2.4. Gingival index (GI)

The Gingival Index had been used to evaluate gum health. It included four partsdthe disto-facial papilla, the facial margin, the mesio-facial papilla, and the entire lingual gingival margindthat made up the tissues surrounding each tooth. The following scale was used to evaluate the degree of inflammation.

0 ¼ Normal gingiva.

1 ¼ The patient has mild inflammation, a minor alteration in colour, slight swelling, and no bleeding upon probing.

2 ¼ The patient exhibits moderate inflammation, redness, swelling plus shiny appearance of the affected area, along with bleeding when probed.

3 ¼ The individual is experiencing severe inflammation, characterized by pronounced redness and swelling. Additionally, there is ulceration present, and a predisposition for spontaneous bleeding.

Clinical attachment level (CAL):

Clinical attachment level (CAL) refers to the measurement of the distance between the base of the periodontal pocket and the cementeenamel junction. The measurements had been taken at the same position as the point of interest, PD.

2.5. Laser application

The laser disinfection procedure will begin by using a 300 mm noninitiated tip to target the pockets. The tip will be held parallel to the tooth, ensuring lengthy access to each tooth. The laser will be operated in pulsed mode CP2, with a peak power of 1.60 W, average power of 0.80 W, and pulse duration of 1.0 ms, using a pulse interval of 1.0 ms, as seen in Fig. 1.

Specialized protective eyewear was used by both the patient and the dentist. One week after the first treatment was finished, each surface of the pocket was exposed to radiation for 20 s. The therapy was administered biweekly, using a wavelength of 940 ± 10 nm and a power output of 0.8 W (9).

Fig. 1. The deployment of a laser beam in a clinical setting.

2.6. Salivary sample collection

A volume of 2 ml of unstimulated saliva will be collected using the spitting technique. The participants will be instructed to refrain from consuming food or beverages, as well as from cleaning and flossing their teeth, for 90 min before collecting the unstimulated salivary samples. Next, the participants will collect their saliva and transfer it into 15 ml test tubes with covers. These tubes will then be placed within sealed flasks with a regulated temperature range of 0e4 °C, as seen in Fig. 2.

The flasks will be promptly brought to the laboratory and thereafter subjected to centrifugation for 5 min. The liquid portion will be gathered and transferred into little tubes known as microtubes. Before testing, the specimens will be stored at a temperature of —80 °C in the laboratory [10].

2.7. Bio-chemical evaluation

Salivary samples were obtained from individuals at two appointments: baseline and three months later. The iNOS concentration was quantified using enzyme-linked immunosorbent assay as seen in Fig. 2 [8,11].

3. Results

3.1. Clinical evaluation outcomes

Following their completion of the research, all participating studied cases underwent follow-up evaluations. The mean and standard deviation values of the values had been displayed. The ShapiroeWilk test of normalcy was used to examine the data for normalcy. ShapiroeWilk findings showed that the data had been regularly distributed (parametric data), hence one-way ANOVA was used for the pairwise comparison, followed by the Post Hoc test.

Fig. 2. Salivary samples stored in falcon tubes.

A significant threshold of *P* less than or equal to 0.05 had been established. The statistical programme SPSS had been used to conduct the analysis (version 25, IBM Colorado, USA).

In the young patients, the nonsurgical groups achieved the highest mean of CAL compared with the laser group at 4 weeks as well as 6 months, however, the Laser achieved the highest mean at Baseline and 3 months while in the Old patients, the nonsurgical groups achieved the highest mean of CAL compared with the laser group at 4 weeks, 3 months along with 6 months, however, the Laser achieved the highest mean at Baseline but *For both subgroups* difference among the two groups was not statistically significant ($P > 0.05$). According to the Independent T-test as seen in Table 1 and Fig. 3.

The impact of treatment type (nonsurgical and Laser) on the iNOS biomarker concentration (u/l) under the same time interval and same patient age (intergroup comparison):

3.2. Before treatment

For both subgroups (young and old patients): While the nonsurgical groups had a higher average concentration of iNOS relative to the laser group, the difference among the two groups was not statistically significant ($P > 0.05$).

3.3. After treatment

In the young age group, in terms of average iNOS concentration, the laser groups outperformed the control group that had no surgery. Although the Laser groups in the old patients group achieved the highest mean iNOS concentration compared with the nonsurgical group, there was a statistically insignificant difference between the two groups ($P > 0.05$) based on the Independent *T*-test, which indicated a statistically significant difference among the two group as seen in Table 2 and Fig. 4.

4. Discussion

Periodontal disease occurs when the immune system overreacts to microbial biofilms. Chronic inflammation develops when the immune system becomes imbalanced due to this aberrant reaction, which triggers cells to perpetually create chemokines and cytokines that promote inflammation.

Table 1. Mean ± SD and intra group comparison of clinical attachment loss (mm) among the two times intervals (before and after) and the two ages (young and old) for both groups.

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	Young		*P* value*	Old		*P* value*
	nonsurgical	Laser		Nonsurgical	laser	
Baseline	3.53 ± 0.36	3.77 ± 0.28	0.286^{NS}	3.77 ± 0.1 9	3.8 ± 0.19	0.828^{NS}
4 weeks	2.6 ± 0.35	2.5 ± 0.33	0.654^{NS}	2.77 ± 0.3 3	2.47 ± 0.4 6	0.269^{NS}
3 month s	2 ± 0.24	1.77 ± 0.4	0.295^{NS}	2.23 ± 0.1 5	1.9 ± 0.62	0.276^{NS}
6 month s	1.8 ± 0.3	1.6 ± 0.37	0.371^{NS}	2.1 ± 0.15	1.73 ± 0.3 7	0.071^{NS}

**P* value for intragroup comparison(nonsurgical vs. Laser) from Independent *T*-test.

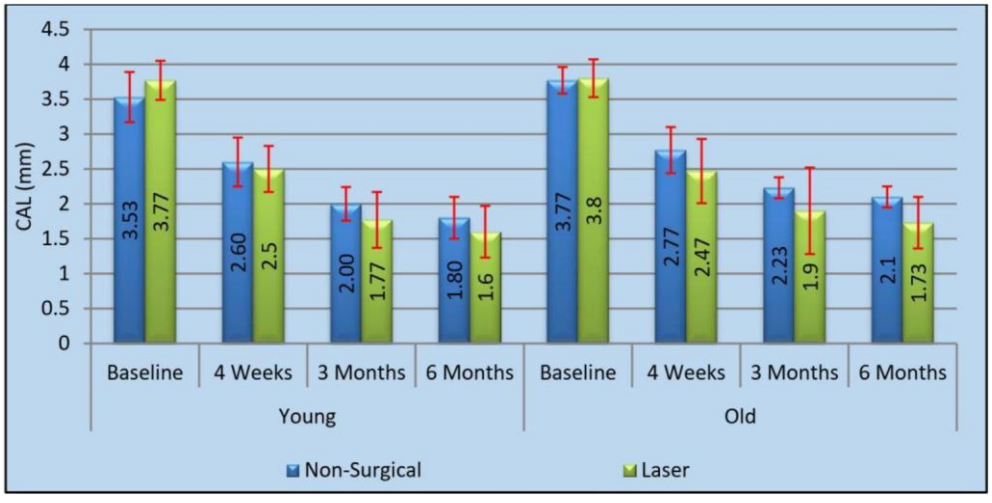

Fig. 3. Bar chart representing Mean and SD of Clinical attachment level at the two-time intervals (before and after) and for the two ages (young and old) for both groups.

Table 2. Mean ± SD and inter-group comparison of inducible nitric oxide synthase biomarker concentration (u/l) between the two groups (nonsurgical and Laser) at the two-time intervals (before and after) for the two patient ages.

	Before		After	
	Young	Old	Young	Old
Nonsurgical	49.15 ± 10.14	42.33 ± 6.35	39.41 ± 10.66	42.66 ± 10.46
Laser	43.97 ± 14.36	39.43 ± 8.57	50.17 ± 14.44	44.34 ± 13.16
P value*	0.269^{NS}	0.621^{NS}	0.026^{S}	0.970^{NS}

**P* value for inter-group comparison (nonsurgical vs. laser) from Independent *T*-Test. eS ¼ statistically significant *P* less than or equal to 0.05 eNS ¼ nonsignificant *P* less than 0.05.

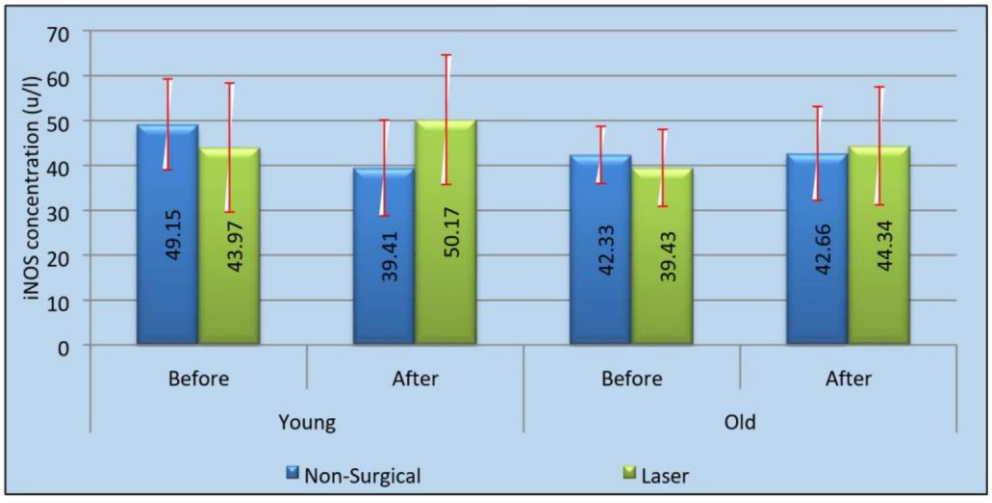

Fig. 4. Bar chart representing Mean and SD of inducible nitric oxide synthasebiomarker concentration (u/l) at the two-time intervals (before and after) and for the two ages (young and old) for both groups.

This inflammation causes an overproduction of free radicals, which in turn increases oxidative stress and may decrease antioxidant capacity [12,13].

Host tissue damage, cellular ageing, and cell death may result from elevated levels of oxidative stress and the overproduction of pro-inflammatory cytokines. Thus, it is essential to treat periodontal disease by regulating oxidative stress levels and limiting the generation of proinflammatory cytokines [14e16].

Whenever an endogenous chromophore or photoreceptor absorbs the energy of photons, a non-thermal process called photo-biomodulation takes place. This absorption causes photophysical and photochemical processes at the molecular and cellular levels. Some of the beneficial benefits of photo-biomodulation that have been studied include lowering inflammatory levels and decreasing oxidative stress [17,18].

In our present study, low concentration of iNOS in young age after nonsurgical periodontal treatment could be explained that NO production increases throughout the deposition of plaques, which might be an early host defense mechanism against bacterial proliferation. Matrix breakdown, including proteoglycan and collagen production reduction, also upregulation of metalloproteinases activity, is known to be accelerated by NO [19,20].

It seems that NO is involved in the immuno-inflammatory process to a significant degree. As a result, periodontitis therapy may include modulating this mediator. Possible new and exciting options for periodontitis therapy include blocking NO generation selectively and using anti-oxidant chemicals to neutralize reactive species or scavenge peroxynitrite [10].

Consistent with research, we discovered a high concentration of iNOS in the laser-treated groups of young individuals in our current investigation. Laser therapy improved AMPK (AMP activated protein kinase) phosphorylation and SIRT3 expression, according to his findings. All the effects might be countered by compound C. The laser-induced downregulation of COX2 (Cyclooxygenase), iNOS, and ROS (reactive oxygen species) was prevented by siRNA-mediated silencing of AMPK or SIRT3 (NAD-dependent deacetylase sirtuin-3). When AMPK was silenced, SIRT3 was downregulated. An increase in nitrite ions, the quantity of nitrites and nitrates, an increase in peroxynitrite synthesis

against a background of decreased levels of hydrogen sulphide and L-arginine, as well as inhibition of arginase activity are among the changes in oral fluid that occur with age [21].

It was found that high concentrations of Inos in both treated groups as people got older. Hydrogen sulphide, in addition to peroxynitrite concentrations (reverse, strong), total nitrate levels (direct, strong), and peroxynitrite content (direct, medium strength) all show robust associations, confirming the findings [22,23].

4.1. Conclusion

This clinical study illustrated that the old age group increases iNOS which can predict a bad prognosis of gingival inflammation, this study also showed that scaling and root planing in combination with low-level laser therapy in Periodontitis stage II was effective at the young age group but ineffective in the old age group.

4.2. Recommendation

Further studies should be conducted with a larger sample size to ensure the oxi-inflammaging theory.

Ethics approval

The ethics information mentioned in the first part: This study has been approved by the study Ethical Committee (REC) of the Faculty of Dental Medicine for girls at Al-Azhar University. (a) Code of approval (REC-ME-24-01). (b) Clinical Trial.gov ID (NCT06140394).

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Consent for publication

Written informed consent was waived by the Institutional Review Board.

Authors biography

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Conflict of interest

There are no conflicts of interest.

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