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A comparative evaluation between salivary periostin level of healthy and chronic periodontitis patients and assessment after scaling and root planing along with low level laser therapy - A clinico - biochemical study

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Abstract

The aim of this study was to evaluate and compare the Periostin level in saliva of patients with healthy periodontium and chronic periodontitis along with its estimation after low level laser therapy (LLLT) as an adjunct to scaling and root planning (SRP). A total of 90 individuals selected were divided into groups I healthy (n = 30), group IIA chronic periodontitis treated with SRP (n = 30) and group IIB chronic periodontitis treated with SRP+LLLT (n = 30). Saliva samples were collected at baseline and at 90th day and was determined using the enzyme-linked immunosorbent assay. The clinical parameter were recorded on baseline,30th day, 90th day and 180th day. At all-time points, the LLLT group showed significantly more improvement in Plaque index (PI), Gingival index (GI), clinical attachment level (CAL) and probing depth (PPD) levels compared to the control group ($P < 0.05$)

Summary: The Periostin level in saliva decreased proportionally with the progression and severity of periodontal disease and negatively correlated with the clinical parameters. Within the limits of the study, this novel biomarker in saliva can be considered a reliable

marker in the diagnosis of periodontal disease susceptibility and activity.

Keywords: Enzyme-linked immunosorbent assay; saliva; periostin; chronic periodontitis; low level laser therapy.

Introduction

Periodontal disease results from a complex interplay between the subgingival biofilm and the host immune-inflammatory events that develop in the gingival and periodontal tissues in response to the challenge presented by the bacteria. The net result of the inflammatory changes is breakdown of the fibers of the periodontal ligament, resulting in clinical loss of attachment, together with resorption of the alveolar bone.[1]

Diagnosis of periodontal disease has been primarily based upon clinical and radiographic measures of periodontal destruction. Alveolar bone, periodontal attachment loss cannot be used to predict the susceptibility to future disease progression or to dictate appropriate treatment plans. Therefore, the drawback of traditionally used diagnostic procedures is that they cannot reliably identify susceptible individuals or distinguish between disease active and inactive sites.[2]There is a need for the development of new diagnostic tests that can determine the presence of current disease activity, predict sites vulnerable for future breakdown, and assess the response to periodontal interventions. A new paradigm for periodontal diagnosis would ultimately affect the improved clinical management of periodontitis.[3]

Periostin, originally termed as osteoblast specific factor-2, when first isolated from the mouse MC3T3-E1 osteoblastic cell line.4 It is a matricellular protein belong to fasciclin-1 family. POSTN secreted by fibroblasts, is found to be present in various tissues, serum, saliva and also in GCF. POSTN levels have been found to decrease in relation to the progression and severity of CP.5 Periostin has been shown to be an important regulator of bone formation.[6] Despite its preliminary description in bone, its biological functions are also essential for connective tissue integrity in both health and disease.[7] Periostin is essential for tissue integrity and maturation, and has a key role as modulator of PDL homeostasis. Release of periostin from human gingival fibroblast is induced by transforming growth factor-beta (TGF-β) and bone morphogenic protein-2. Its expression is downregulated in fibroblasts when exposed to tumor necrosis factor-alpha (TNF-α) and Porphyromonas gingival is lipopolysaccharide both of which are abundantly seen in periodontitis.[8]

Applying lasers as an adjunctive or alternative to conventional mechanical treatment had a great run in the treatment of gingival inflammation. LLLT is recommended for its pain-reducing, wound healing promoter and anti-inflammatory effects.[9] It causes fibroblast proliferation, maturation and stimulates the production of basic fibroblast growth factor (bFGF), reduces plaque levels, gingival inflammation, enhances wound healing, and increases bone deposition.[10]

Materials and methods

Subject Selection, Study Design, and Clinical Procedures

A total of ninety patients (54 men, 46 women), 30 healthy and 30 chronic periodontitis patients who were classified as moderate to advanced chronic periodontitis periodontitis according to the 1999 American Academy of Periodontology workshop were included in the study. Patients selected from the Outpatient Department of Periodontology, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh. Written informed consent was obtained from all subjects.

Patients with age group of 25-55 years of either sex, systemically healthy with no contraindication to periodontal surgery, having probing pocket depth ≥ 5 mm with minimum of 20 natural teeth remaining in the oral cavity were included in the study. Patients exhibiting any of the following criteria were excluded: (1) Patients with history of any systemic disease (Diabetes mellitus, cancer, bone and collagen metabolic diseases or disorder that compromise Periostin level). (2) Subjects taking medications, such as corticosteroids, calcium channel blockers or immunosuppressive drugs, which are known to interfere with periodontal wound healing, (3) Allergic to medications, (4) Current pregnancy or lactation, (5) Smokers /Tobacco users, (6) Poor compliance or failure to maintain good oral hygiene, (7) Past periodontal therapy.

Patients enrolled for the study were randomly divided into 3 main groups, according to the type of treatment rendered to them as follows: Group I- patients with healthy periodontium (n=30), Group IIA– patients who received only SRP (n=30), Group IIB - consisted of patients who received LLLT after SRP (n=30). All patients received oral hygiene instructions and supragingival scaling in two appointments one week apart before treatment and subgingival SRP were done in a single appointment for each patient in all groups using hand instruments and ultrasonic devices. Laser therapy was performed thrice to the LLLT group on the first, second, and seventh days after SRP. Laser treatment was performed using a gallium aluminium-arsenide (GaAlAs) diode laser. The physical parameters of this unit used during the treatment were as follows: wavelength 980 nm; Average output, 0.5 W; with an Energy Density of 4-5 J/cm2 and in continuous wave output.[9] Laser was used in non-contact mode directing towards gingival sulcus for 20 seconds, over each surface covering the entire oral cavity. The application distance was 0.5 to 1 cm because this distance difference did not affect the spot size with the hand piece that was used.9 (figure-1)

Plaque index (PI), Gingival index (GI), Probing pocket depth (PPD), Clinical attachment level (CAL) were recorded prior to the commencement of the study at baseline for all the 3 groups, and at 30th day, 90th day and 180th day for group IIA and group IIB. All subjects were examined by one examiner using UNC-15 Periodontal Probe. Radiographic evaluation was done at baseline by orthopantomogram (OPG).

Saliva sampling

Whole saliva was collected at baseline from all the patients. Patients were requested to refrain from eating and drinking for at least 1 hour prior to saliva collection. Patients was seated comfortably with eyes open, head tilted slightly forward, and instructed to rest for 5 minutes. Using the Draining method (Navazesh et al 1993)115 ml of unstimulated saliva was collected in ice-cooled sterile collection tube. Saliva samples was centrifuged for 20 minutes at 2000-3000 rpm/min. After collecting the supernatant carefully, the sample was stored at -20 degrees in low temperature freezer to avoid loss of bioactivity and contamination until further processing.

Biochemical Analyses

Saliva samples were analyzed for Periostin using commercially available sandwich enzyme linked immunosorbent assays (CHONGQING BIOSPES®) according to the manufacturer's instructions. The concentrations were measured at a wavelength of 450 nm. Results were reported as total amounts of Periostin in saliva. (Figure 2-5)

Statistical Analyses

The data collected were analyzed using a statistical software package (SPSS, version 16.0). Independent student t test was used for the comparison of mean values for same variables between two samples. The paired-sample t test was used to assess the statistical significance of differences between the baseline, 30th day, 90thdayand180thday within each group. Intergroup comparison of means between group I, group IIA & group IIB was found using ANOVA with Tukey's post-hoc test. Statistical significance was set at the 99% confidence level (P <0.01) and at the 95% confidence level (P <0.05) Significance is assessed at 5% level of significance.

Results

All patients included in the evaluation completed the 6-month study period and none missed any appointments. Healing was uneventful in all cases. No adverse effects related to the laser irradiation have been reported.

Clinical Assessments

The clinical and biochemical parameters showed improvement throughout the study period. The clinical results of the study are summarized in Table 1. There were no statistically significant differences in PI, GI, PD, and CAL between the groups at baseline (P >0.01). The results of clinical measurements (mean – SD) and differences between baseline and at different time interval are displayed in Table 1. In both groups (IIA and IIB), all clinical parameters showed statistically significant reduction between baseline and all time points. The PI, GI, PPD and CAL reduction were significantly higher in the LLLT group as compared to different time intervals (P <0.001).

Biochemical Assessments

Differences in the Periostin level at different time interval between the groups are presented in Table 2 (a) and 2(b). Intergroup comparison of mean Periostin level at baseline among group I, IIA &IIB showed a significant difference. (P- 0.001) and statistically non-significant difference (P-0.6) between group IIA & IIB was found using ANOVA with Tukey's post-hoc test. Significant change in Periostin level between the groups at 90th day was statistically highly significant (0.0001) with Group II B (SRP+LLLT) showing a greater change in Periostin level.

Discussion

In periodontics, traditional clinical criteria are often insufficient for determining sites of active disease, for monitoring quantitatively the response to therapy or for measuring the degree of susceptibility to future disease progression.3 Biomarkers, whether produced by normal healthy individuals or by individuals affected by specific systemic diseases, are tell-tale molecules that could be used to monitor health status. Informative biomarkers can further serve as early sentinels of disease, and this has been considered as the most promising alternative to classic environmental epidemiology.[12]

Periostin (POSTN, PN, or osteoblast specific factor OSF-2) is a novel biomarker in connection with pathogenesis of periodontitis. The matricellular protein periostin is strongly expressed in collagenrich connective tissues such as periodontal ligaments (PDLs), skeletal muscle, adipose tissue, tendons, skin, and bone. It is localized in the periosteum and PDL, where it is seen in the cytoplasmic extensions of the PDL fibroblasts. It mediates and augments collagen fibrillogenesis, cell migration, adhesion, response to mechanical stress, and wound healing. The molecular mechanism of periostin action in collagen crosslinking has been investigated, and the results showed that periostin interacts with BMP-1 and enhances the proteolytic activation of lysyl oxidase (LOX), an enzyme

responsible for cross-link formation. 13 Together with tenascin-c, periostin acts as a scaffold that increases the deposition of BMP-1 into the fibronectin matrix to activate pro-LOX. The periostin stabilizes the precursor LOX, which then gets proteolyzed to LOX. Thus, periostin aids in the formation of high stiffness collagen through effective collagen cross-linking.[14]

Exposure to pro-inflammatory cytokines (TNF-a) and/or microbial virulence factors (Porphyromonas gingival is lipopolysaccharides) initially increases periostin levels in PDL fibroblasts, but chronic exposure leads to its decrease.8 During the disease process, the well-organized PDL is converted to an inflamed tissue, thereby reducing the periostin levels. Since periostin is expressed only by PDL fibroblasts, any decrease in its level reflects a loss of PDL tissue integrity.14 Periostin may be involved in healing after periodontal surgery, regenerative procedures, or even after placement of dental implants. By promoting the migration of fibroblasts14 and osteoblasts. [15]

In our study, all the assayed samples of saliva showed the presence of periostin. Periostin level significantly reduced in chronic periodontitis patients when compared to healthy individuals ($P < 0.001$). This is in agreement with the studies.[5,10,16,17,18] Balli et al.5analyzed GCF and serum periostin levels in healthy, gingivitis and CP patients and concluded that GCF periostin concentration decreased with the periodontal disease severity.[5] In periodontitis patients, level of periostin decreases due to two reasons. Firstly, bacterial competition can cause a reduction in level of periostin produced by PDL fibroblasts and secondly, due to reduction in number of PDL fibroblasts. Reduction in the level of periostin compromises PDL stability and increases the risk of subsequent damage and inflammatory process due to reduction in biomechanical and structural potential of PDL.[18]

At 90 days after treatment of chronic periodontitis, periostin level was increased when compared to the baseline in both Group IIA and IIB. Moreover, with greater increase in LLLT group. ($P < 0.001$) Perhaps this may be due to the effects of LLLT causing a reduction in Pg. and TNF-α both of which decrease the expression of periostin. This is in accordance with study by PA dial-Molina et al.[17] The present study results, therefore, confirmed a negative correlation between saliva POSTN levels and periodontal diseases. Therefore, the reduction in POSTN levels seen in CP might suggest that this novel molecule takes part in maintaining normal periodontal tissue function9 and the high levels of POSTN in saliva of healthy patients indicates its importance in maintenance of integrity of tissues, which is in accordance with the study conducted by Aral et al.16, Balli et al.[5], Baeza et al.[19]

LLLT is also known as soft laser therapy or biostimulation.20 The bio stimulatory and inhibitory effects of LLLT are governed by the Arndt-Schulz law. According to this law, low-dose will increase physiologic processes, and strong stimuli will inhibit physiological activity. The output powers for LLLT range from 50 to 500 mW with wavelengths in the red and near infrared of the electromagnetic spectrum, from 630 to 980 nm with pulsed or continuous-wave emission. It does not cause temperature elevation within the tissue, but rather produce their effects from photo bio stimulation effect within the tissues. LLLT do not cut or ablate the tissue. [21]

This interaction might be affected by some parameters, such as wavelength, power, energy density, treatment duration, method of application and condition of tissue. The dose applied is one of the important treatment

parameters to benefit from LLLT. Bio stimulation has been reported in the literature with doses between 0.001 and 10 J/cm2 as a therapeutic window.9 Even though applied dose is in the therapeutic window range; it might be too low or too high for the desired effect. Mester et al.22 suggested that doses of 1 to 2 J/cm2 are necessary to see an effect on wound healing. As the wavelength of the diode laser is absorbed by protohemin and protoporphyrin IX pigments of the pigmented anaerobic periopathogens which lead to vaporization of water and causes lysis of the cell wall of the bacteria, leading to bacterial cell death.[23] In addition LLLT eliminates the antimicrobial's problems like resistance, allergy and side effects thus can be used even in children and pregnant women.[24,25]

In our study we used a GaAlAs diode laser with a wavelength of 980 nm, output power of 0.5 W, with an energy density 4-5 J/cm2 and in continuous wave output mode on the first, second, and seventh days after the treatment. This dose has also been proved to enhance epithelialization and wound healing by previous studies after gingivectomy and gingivoplasty.26 There is well documented evidence to show that multiple irradiations are more effective than a single dose, which is a factor in bone formation and fibroblast growth.[27]

The results of our study show that there is a statistically significant improvement in clinical parameters after non-surgical periodontal treatment in each group. Oral hygiene and gingival health was assessed using the PI and GI respectively. Over the period of 180 days, the improvement in terms of mean difference of PI, GI scores is significantly more in the LLLT group (IIB) compared to the IIA. The greater reduction in GI in the Group IIB is probably justified by the antibacterial and anti-inflammatory effects of the LLLT and in accordance with the findings of other studies. [28,29,30] Nonetheless, in the studies conducted by Makhlouf et al.[31] they found statistically non-significant GI differences.

LLLT after non-surgical periodontal treatment resulted in significantly more reduction of PPD compared to the group IIA (SRP alone). In harmony with the results obtained in the previous studies.[9,10,29,31,32] Moritz et al. shown that irradiation with the diode laser facilitates considerable bacterial elimination, especially of A. Actino my cetemcomitans, Prevotella intermedia, Porphyromonas gingival is, and proinflammatory cytokines.[33] Sakuri et al. described that low energy laser irradiation significantly inhibit prostaglandin E2 (PGE2) production that was stimulated by lipopolysaccharides (LPS) in human gingival fibroblast (hGF) cells through a reduction of cyclooxygenase-2 gene expression indicating the possible anti-inflammatory role of diode laser which may have a therapeutic effect on periodontitis.[34] On a cellular level, metabolism is increased, due to bio stimulation caused by diode laser thus increasing the production of adenosine triphosphate, the fuel that powers the cell. This increase in energy is available to normalize cell function and promote tissue healing.21 Contrary to our findings, Lai et al. suggested that low-power laser did not result in any additional clinical benefit.[35]

The findings of this study are in accordance with various studies [9,36,37,30] where application of LLLT adjuvant to SRP yielded significant results in improvement of the CAL. This may be due to additional effect of Low-level laser therapy on fibroblasts by promoting proliferation and increasing cell numbers, secretion of growth factors and differentiation of fibroblasts into myofibroblasts.[38,39] Hence, the findings of the present study may bring up the proposal that averting the depletion of POSTN or increasing the levels of POSTN may be helpful in repairing the tissues at a faster rate and increasing

attachment gain. Moreover, additional treatment with LLLT improves clinical parameters and increases periostin levels in CP patients. Even though there were statistical differences between the groups, the clinical improvement observed was rather minor. Thus, within its limits, further studies are required to assess the efficacy of this biomarker for early detection of periodontal disease and prevention of its progression and to establish the efficacy of LLLT in chronic periodontitis patients.

Conclusion

When the results in the present study are considered in conjunction with those of previous reports, it is concluded that the POSTN level in saliva can be considered as a reliable marker in periodontal disease diagnosis, disease activity, and healing. Additionally, chair side diagnostic tests and periostin specific therapeutic strategies could be developed to reduce and arrest further periodontitis associated alveolar bone destruction.

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Legend Figures

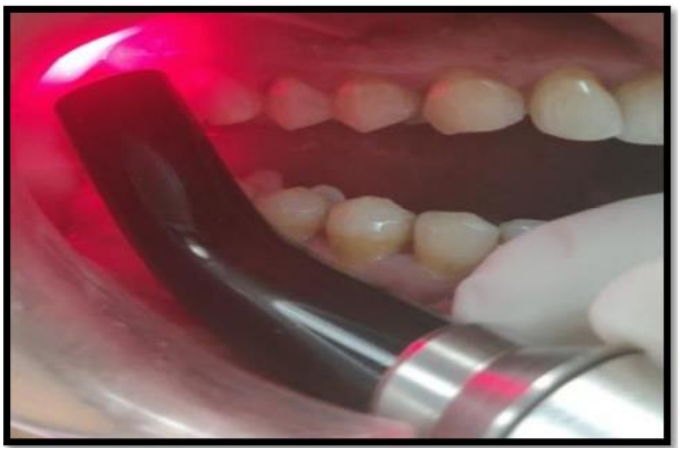

Figure 1: Application of Low-Level Laser Therapy.

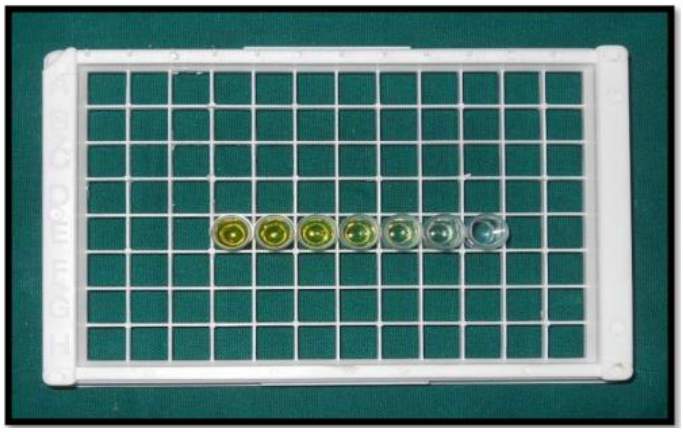

Figure 2: Colour change in standard wells.

Figure 3: After addition of antibody solution into wells.

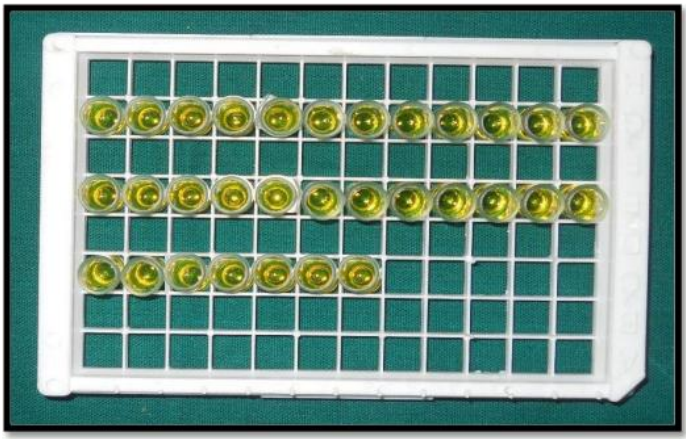

Figure 4: Colour change in wells with saliva samples of healthy patients.

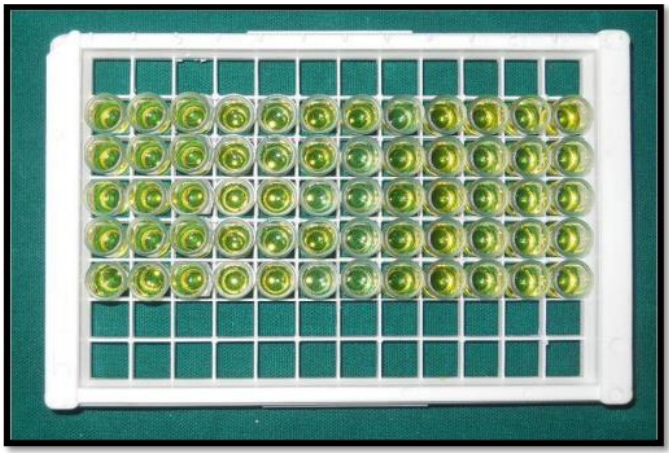

Figure 5: Colour change in wells with saliva samples of chronic periodontitis patients.

Legend Tables

Table 1: Differences in Clinical Parameters

PARAMETER		BASELINE	30th DAY	90th DAY	180th DAY	P VALUE
PI	GROUP IIA	2.05±0.32	0.86±0.08	0.80±0.10	0.79±0.12	0.001(H.S) *
	GROUP IIB	2.17±0.16	0.81±0.09	0.74±0.09	0.73±0.10	0.001 (H.S)
	P VALUE	**0.068 (N.S)†**	**0.02(S)‡**	**0.017(S)**	**0.03(S)**	
GI	GROUP IIA	1.83±0.28	0.98±0.12	0.88±0.20	0.84±0.15	0.001 (H.S)
	GROUP IIB	1.95±0.18	0.92±0.09	0.78±0.18	0.76±0.14	0.001 (H.S)
	P VALUE	**0.055 (N.S)**	**0.032 (S)**	**0.04 (S)**	**0.03 (S)**	
PPD	GROUP IIA	5.50±0.62	5.03±0.71	3.53±0.57	2.98±0.36	0.001 (H.S)
	GROUP IIB	5.63±0.71	4.71±0.50	3.20±0.55	2.75±0.50	0.001 (H.S)
	P VALUE	**0.453 (N.S)**	**0.04 (S)**	**0.02 (S)**	**0.04 (S)**	
CAL	GROUP IIA	6.13±0.86	5.43±0.56	4.63±0.66	3.60±0.61	0.001 (H.S)
	GROUP IIB	6.40±0.96	4.99±0.72	4.08±0.92	3.29±0.48	0.001 (H.S)
	P VALUE	**0.264 (N.S)**	**0.01 (S)**	**0.01 (S)**	**0.03 (S)**	

*Highly Significant, † Non-Significant, ‡ Significant.

Table 2: Differences in Biochemical Parameter.

Table 2(a): Intergroup comparison of mean Periostin Level at Baseline among group I, group IIA & group IIB

Group	N	Mean	SD	F value	*p*-Value
Group I	30	27.96	1.97	263.55	**0.001 (H.S)**
Group IIA	30	17.86	2.22		
Group IIB	30	17.43	1.81		

Table 2(b): Intergroup comparison of mean Periostin Level between various time intervals

Time interval	Group	Mean	SD	Mean difference	t- value	p-value
Baseline	**IIA**	17.86	2.22	0.433	0.827	**0.412 (N.S)**
	IIB	17.43	1.81			
90th day	**IIA**	23.53	2.22	-3.04	-4.97	**0.0001 (HS)**
	IIB	26.57	2.44			