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EFFECTS OF LOW LEVEL LASER THERAPY (LLLT) BIOSTIMULATION ON GCF LEVELS OF SCLEROSTIN IN SURGICAL MANAGEMENT OF INTRABONY DEFECTS

Periodontology

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ABSTRACT

Aim: To evaluate the effects of low level laser therapy (LLLT) biostimulation following open flap debridement (OFD) and correlate with GCF levels of sclerostin in intrabony defect management.

Materials and methods: 30 patients with 2 and 3 wall intrabony defects were treated using LLLT biostimulation along with OFD (n=15) and OFD alone (n=15). Patients were recalled at 3 and 6 months to assess the changes in sclerostin levels, Plaque index, Gingival index, Probing pocket depth, Clinical attachment level, Defect depth and gain in defect height.

Results: Both test and control groups showed a statistically significant improvement in all clinical parameters, sclerostin biomarker levels and defect depth from baseline to 3 and 6 months. However, the test group had a statistically significant reduction in defect depth at 3 to 6 months and the sclerostin biomarker levels also showed a statistically significant correlation to defect depth at all-time intervals.

Conclusion: Within limitations of the present study it can be concluded that LLLT has a greater benefit over OFD alone in intra bony defect regeneration as observed by progressive bone fill from 3 to 6 months.

KEYWORDS

GCF, Lasers, Fibroblasts, Bone Regeneration

Introduction:

Periodontal disease occurs due to imbalance between microbial challenge and host response, leading to periodontal destruction and bone loss[1,2]. Bone metabolism is regulated by diverse signal transduction pathways[3,4]. One pathway is osteoclast-mediated bone resorption which is closely related to interaction of the TNF superfamily: receptor activator of nuclear factor-κB ligand (RANKL); its receptor, RANK; and its decoy receptor Osteoprotegerin (OPG)[4]. RANKL binds directly to RANK on osteoclast lineage cells, resulting in osteoclast differentiation and activation thereby initiating bone resorption. RANKL activity is regulated by OPG by binding to it and preventing it from binding to RANK, thereby inhibiting osteoclastogenesis and bone resorption[3-5]. Other pathway is by regulating the Wnt signalling, which increases bone formation and regeneration via stimulation of osteoblast development [6-9].

Sclerostin, a product of the *SOST* gene, produced by osteocytes is a secreted glycoprotein that binds low-density lipoprotein receptor-related protein 5, 6 [10,11,12]. Its expression, suppresses osteoblastogenesis and reduces the viability of osteoblasts and osteocytes, leading to bone resorption by blocking Wnt and bone morphogenetic protein signalling (BMP- 4 and 7)[13,14]. Sclerostin also promotes osteoclast formation via RANKL-dependent pathway as well as by interacting with osteoblasts[15]. Preliminary evidence for reliability of sclerostin as a bone marker from various *in vitro and in vivo* animal studies which revealed that increase in sclerostin levels causes supressed bone formation [16-19]. Recently in a human study, sclerostin when compared to OPG/RANKL as a marker for bone mineralization has been found more reliable in chronic periodontitis patients compared to healthy controls[20].

Open flap debridement (OFD) has been traditionally used for the treatment of intrabony defects[21-24].OFD alone without combination of regenerative materials also results in significant clinical and radiographic benefits[24-28]. Even though OFD alone can produce favourable results in intrabony defect regeneration, there was a necessity to overcome the disadvantages of OFD such as pain, discomfort and delayed post-operative wound healing[24,25].

In this context low level laser therapy (LLLT) has introduced to overcome this negative effects. Several *in vitro* animal[29-38] and human studies[39-53] have shown that low level laser therapy (LLLT) demonstrated superior benefits on stimulating bone formation, cellular proliferation and alkaline phosphatase activity which leads to increase in bone formation [54, 55]. Recent meta-analysis reports showed LLLT enhanced osteoblastic cell proliferation and differentiation[56, 40, 42]. This suggests that LLLT by itself is equally effective in stimulating bone regeneration. So far, there have been histological and radiographic studies evaluating intrabony defect regeneration following use of LLLT.

As bone biomarkers reflect the bone healing at a molecular level, bone biomarker based evaluation of intrabony defect regeneration using LLLT will be more evidence based. In this context, sclerostin has been found to be a reliable marker of bone remodelling as evidenced by *in vivo* human studies [12,18,20]. Further, sclerostin as a marker for evaluating bone regeneration following treatment with LLLT in human intrabony defects has not been studied so far. Therefore, the aim of this case-control clinical trial was to evaluate clinical and radiographic outcomes following adjunctive LLLT biostimulation on GCF levels of sclerostin in surgical management of intrabony defects. Thus we hypothesize that use of LLLT biostimulation as an adjunct to OFD might lead to additional benefits as evidenced by reduction in sclerostin levels and gain in defect height.

MATERIALS AND METHODS:

Patients visiting the Department of Periodontology, S.R.M. Dental College and hospital, Chennai were selected for the study. The study which was conducted from April 2016 to August 2017. Patients satisfying the following criteria were selected.

Inclusion and Exclusion Criteria:

The study population consisted of 30 individuals with age range of 30 – 55 years. Patients diagnosed with chronic periodontitis (based on AAP 1999 classification)[57] and individuals having a minimum of 20 permanent teeth with sites ≥ 5 mm of probing pocket depth, ≥ 3mm of clinical attachment loss. Two walled, three walled and combined interdental intra bony defects exhibiting ≥ 3mm of intrabony component as determined by radiographs and intrasurgical exposure were enrolled in the study. Subjects with periapical pathologies, systemic diseases (e.g., diabetes mellitus; human immunodeficiency virus; bone-related diseases; and radiation or immunosuppressive therapy, pregnancy, lactation, smoking within the past five years. No history of periodontal therapy for at least six months. Teeth with mobility and furcation involvement were excluded from the study.

Study Design and sample size calculation, randomization:
The study design was a single blinded randomized case - control clinical trial. All patients were explained about the study protocol and a written informed consent was obtained. The study protocol was approved by the Institutional Scientific and Ethical Review Board (IRB Approval no-SRMDC/IRB/2015/MDS/NO: 508). Sample size calculation was performed based on the results of a previous study done by Gupta M et al 2013[25]. The following formulae were used and the sample size calculation derived using a G POWER VERSION 3.1.9.2. Software. Sample size was calculated based on proportionate methodology with 0.05 α error set at 95% power. The patients who fit the inclusion and exclusion criteria, and who were willing to participate in the study, were enrolled serially from 1 to 30 written in a card and shuffled before surgery. Even numbered registrants were assigned to receive LLLT biostimulation and OFD (Test group/Group I) & Odd numbered registrants were assigned to receive OFD alone (Control group/Group II) however the examiner was unaware of the allocation throughout the study.

Clinical Parameters evaluated were plaque index, gingival index, Sulcular bleeding index, Probing pocket Depth and Clinical Attachment Level were measured at baseline, 3 months & 6 months post surgically. All parameters were recorded by the same blinded examiner.

Radiographic measurements with - RVG (mm) were taken for all patients at baseline, 3 and 6 months. Individually customized silicon bite blocks to index the dentition were attached to metal bar of film holder device using long cone paralleling technique. The PSP plates were mounted on X -ray positioning and holding device, were scanned using DIGORA software and digital images were obtained and evaluated for radiographic defect fill, gain in defect height of osseous defects with a computer assisted method using coral draw software. Radiographic defect depth measurements done by drawing a horizontal line connecting the interproximal CEJ's of adjacent teeth corresponding to the defect site, which was marked as point A on mesial CEJ of one tooth and point B on the distal CEJ of other tooth respectively. A perpendicular line is drawn from point A to the base of the defect, which is marked as point D. Another perpendicular line is drawn from point B to the crest of defect, which is marked as point C. Defect depth measurements were calculated in mm as (fig no: 1).

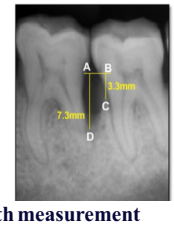

Fig no:1 Defect depth measurement
Distance from A to D, which signifies the intrabony defect depth measurement.

Gain in defect height (mm) was calculated from the defect depth measurements using the formula:

(A TO D) BASELINE – (A TO D) 3 OR 6 MONTHS

Based on the clinical and radiographic investigations the defect sites were identified and selected for GCF sample collection. A total of 90 GCF samples were collected from subjects with two & three wall intrabony defects from 6 sites of the corresponding defect site. The collected GCF samples were expelled from the micro-capillary pipette with a jet of air using a blower immediately into an air tight plastic eppendorf tubes and stored at -80˚C until assayed. The collection of GCF was done at subsequent visits 3 & 6 months. ELISA kit for quantification of human SOST levels in GCF was purchased from Korain Biotech, Shanghai, China (fig no: 2). Assay was performed according to the manufacturer's recommendations.

Fig no: 2 Human sclerostin ELISA kit

SURGICAL PROCEDURE (Test and Control Fig no: 3, 4)
Patients were instructed to rinse for 30 seconds with 0.2% chlorhexidine gluconate solution. After proper pre surgical preparation, the operative site was anaesthetized following administration of 2% lignocaine hydrochloride with 1:80000 adrenaline. In both the groups a full thickness mucoperiosteal flap was elevated to visualize and optimize defect debridement and ultrasonic scalers, gracey curettes were used to debride the root surface followed by copious saline irrigation and proper isolation was done. Intra surgical assessment – The defect morphology and the number of walls present were evaluated and confirmed for the presence of two and three wall defects with ≥ 3 mm. Additionally, in the TEST GROUP a low lever laser therapy (LLLT) biostimulation was performed with 810 nm diode laser in continuous non-contact mode with a setting of 0.8-1.0 watts for 5 sec on both the facial and the palatal/lingual aspects of the defect site. In both the groups flaps were approximated using 3-0 Merssilk sutures by simple interrupted direct loop suturing technique. The surgical site was protected with a non-eugenol periodontal dressing (Coe pack). Antibiotics and analgesics were prescribed postoperatively. Patients were advised to rinse twice daily with chlorhexidine gluconate from the day following the surgery and to discontinue use of other oral hygiene measures at the surgical site for two weeks. All the patients were re-called for review and after removing the periodontal dressing, the site was irrigated with normal saline after suture removal and there on patients were reviewed at 3 months and 6 months for clinical, radiographic parameters and GCF biomarker levels.

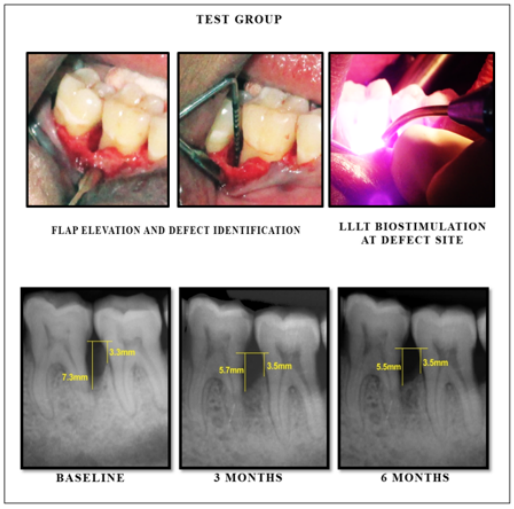

Fig no: 3 Test group

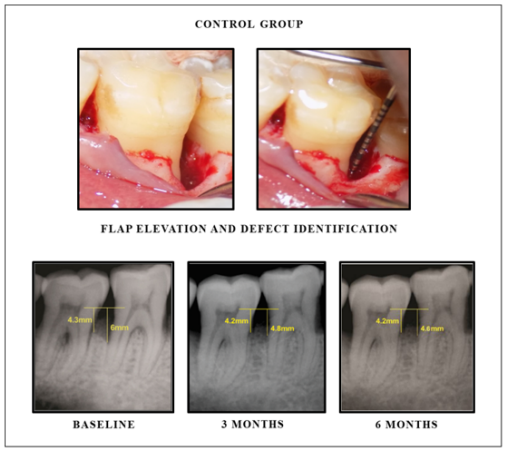

Fig no: 4 Control group

RESULTS:
All clinical, radiographic parameters and biomarker levels were highly significant in test group between various time intervals (table no: 1). In control group clinical and biomarker levels were highly significant between various time intervals except the defect depth and gain in defect height were not significant at 3-6 months (table no: 2). However, GI was highly significant at baseline - 3 months, baseline - 6 months it was not significant at 3-6 months in both test and control groups.

Intergroup comparison showed a statistically significant correlation for biomarker levels at various time intervals (table no: 3)

All clinical parameters showed highly statistically significant correlation for defect depth and sclerostin marker levels at various time intervals in test group. Gain in defect depth has showed a strong negative correlation with sclerostin levels, however the values were not statistically significant in test group (table no: 4). Where as in the control group clinical parameters showed highly significant correlation with sclerostin alone. Only PPD, CAL showed significant correlation with defect depth and the other parameters were not statistically significant (table no: 5).

Table 1: Intra group Comparison of the clinical parameters (BOP, PI and GI) at different time intervals in both test and control groups

Para meters	Baseline–3months				Baseline-6 months				3 months–6 months			
	Test		Control		Test		Control		Test		control	
	Z value	p value	Z value	p value	Z value	p value	Z value	p value	Z value	p value	Z value	p value
BOP	3.46	0.001**	3.37	0.001**	3.42	0.001**	3.42	0.001**	3.43	0.001**	3.449	0.001**
PI	3.53	<0.001**	3.44	0.001**	3.42	0.001**	3.42	0.001**	3.45	0.001**	3.447	0.001**
GI	2.73	0.006**	2.53	0.011*	2.48	0.013*	2.33	0.020*	1.41	0.157	1.890	0.059

(p <0.05 statistically significant, * significant, ** highly significant)

Table 2: Intra group Comparison of the clinical parameters (PPD and CAL), radiographic parameters and biomarker levels at different time intervals in both test and control groups

Parameters	Baseline–3months				Baseline-6 months				3 months–6 months			
	Test		Control		Test		control		Test		control	
	t value	p value	t value	p value	t value	p value	t value	p value	t value	p value	t value	p value
PPD	5.72	<0.001**	5.59	<0.001**	9.40	<0.001**	9.52	<0.001**	7.15	<0.001**	3.84	0.002**
CAL	5.48	<0.001**	3.11	0.008**	9.17	<0.001**	5.19	<0.001**	5.82	<0.001**	4.54	<0.001**
Sclerostin	11.04	<0.001**	8.48	<0.001**	15.32	<0.001**	9.72	<0.001**	13.25	<0.001**	4.61	<0.001**
Defect depth	4.07	0.001**	5.03	0.001**	5.87	<0.001**	5.22	<0.001**	3.44	0.004**	1.87	0.082
Gain in defect height	-	-	-	-	-	-	-	-	3.44	0.004**	1.87	0.082

(p <0.05 statistically significant, * significant, ** highly significant)

Table 3: Inter-group comparison of the clinical, radiographic parameters and biomarker levels between test and control groups

Parameters	Test (Mean ± S.D)	Control (Mean ± S.D)	p-value
BOP- Baseline	1.07 ± 0.25	0.88 ± 0.24	0.054
BOP - 3 months	0.35 ± 0.28	0.32 ± 0.27	0.910
BOP - 6 months	0.12 ± 0.18	0.18 ± 0.22	0.339
GI -Baseline	1.26 ± .328	1.45 ± 0.56	0.541
GI- 3months	0.07 ± 0.17	0.08 ± 0.15	0.475
GI- 6 months	0.00 ± 0.00	0.00 ± 0.00	0.999
PI - Baseline	1.58 ± 0.40	1.57 ±0.52	0.966
PI - 3 months	0.32 ± 0.30	0.32 ± 0.22	0.603
PI - 6 months	0.08 ± 0.18	0.20 ± 0.25	0.067
PPD – baseline	5.76 ± 1.00	5.14 ± 0.81	0.073
PPD – 3 months	3.80 ± 0.55	3.77 ± 0.67	0.904
PPD – 6 months	3.03 ± 0.46	3.32 ± 0.56	0.147
CAL – baseline	4.02 ± 1.57	4.01 ± 1.58	0.984
CAL – 3 months	2.48 ± 1.21	2.48 ± 1.14	0.999
CAL – 6 months	1.68 ± 1.20	1.82 ± 1.14	0.747
Defect depth –Baseline	6.52 ± 1.73	5.76 ± 2.20	0.303
Defect depth – 3 months	5.54 ±1.44	4.75 ± 1.80	0.194
Defect depth - 6 months	4.77 ±1.14	4.69 ±1.85	0.888
Sclerostin (ng/ml)–baseline	25.3 ± 1.88	21.4 ± 1.25	<0.001**
Sclerostin (ng/ml) - 3 months	19.7 ± 1.37	18.5 ± 1.11	0.011*
Sclerostin (ng/ml) - 6 months	15.0 ± 1.69	17.1 ± 0.99	<0.001**
Gain in defect depth - 3 months	0.98 ± 0.93	1.01 ± 0.77	0.91
Gain in defect depth at – 6 months	1.75 ± 1.15	1.07 ± 0.79	0.07

(p <0.05 statistically significant, * significant, ** highly significant)

Table 4: Pearson correlation values between clinical parameters, radiographic parameters and biomarker levels at various time intervals in test group

Parameters	Defect depth (mm)		Sclerostin level (ng/ml)		Gain in Defect Height	
	r-value	p-value	r-value	p-value	r-value	p-value
BOP	0.61	0.00**	0.79	0.00**	-0.20	0.285
PI	0.54	0.00**	0.82	0.00**	-0.14	0.443
GI	0.43	0.003**	0.82	0.00**	-0.01	0.931
PPD	0.54	0.00**	0.75	0.00**	-0.33	0.068
CAL	0.47	0.001**	0.56	0.00**	-0.07	0.703
Defect depth(mm)	1	-	0.35	0.016*	-0.03	0.851
Sclerostin level (ng/ml)	0.358	0.016*	1	-	-0.20	0.280
Gain in Defect Height	-0.03	0.85	-0.204	0.280	1	-

(p <0.05 statistically significant, * significant, ** highly significant)

Table 5: Pearson correlation values between clinical parameters, radiographic parameters and biomarker levels at various time intervals in control group

Parameters	Defect depth (mm)		Sclerostin level (ng/ml)		Gain in Defect Height	
	r-value	p-value	r-value	p-value	r- value	p-value
BOP	0.17	0.25	0.68	0.00**	0.061	0.74
PI	0.27	0.06	0.73	0.00**	0.27	0.14
GI	0.26	0.07	0.74	0.00**	0.02	0.88
PPD	0.35	0.01*	0.67	0.00**	-0.01	0.95
CAL	0.30	0.04*	0.54	0.00**	-0.07	0.68
Defect depth(mm)	1	-	0.08	0.57	0.31	0.09
Sclerostin level (ng/ml)	0.08	0.57	1	-	-0.00	0.96
Gain in Defect Height	0.31	0.09	-0.00	0.96	1	-

(p <0.05 statistically significant, * significant, ** highly significant)

DISCUSSION:

Periodontal regenerative techniques utilizing membranes and grafts have been established to have predictable outcomes even though they have drawbacks such as prolonged operative time, donor graft site morbidity, patient compliance and variable bone regenerative outcomes[58, 59]. In this context, the concept of LLLT biostimulation in intrabony defect regeneration is being considered as an alternative therapeutic modality. Human studies have demonstrated, sclerostin as a marker of mature osteocytic activity and exhibits a negative correlation with alveolar bone remodelling[12, 20]. Since, there are no studies correlating sclerostin with LLLT in human intrabony defect management, the current study was designed to evaluate the sclerostin levels and radiographic bone fill following LLLT biostimulation in human two and three wall intrabony defects.

In the present study BOP, PI, PPD and CAL in both groups showed a statistically significant reduction at all-time intervals ($p < 0.001$) (Table 1, 2). The above findings suggest that use of LLLT + OFD and OFD alone yielded similar reduction in clinical parameters. There are no human studies to compare the outcome following LLLT as an adjunct to OFD however, AboElsaad et al 2009, performed a study to evaluate the effects of LLLT in regeneration of intrabony defects and concluded that there was significant difference between laser and non-laser sites in terms of improvement in PPD and CAL[50].

In the present study sclerostin levels showed a highly significant reduction in both groups when compared at various time intervals ($p < 0.001$) (Table 1, 2). These results were similar to previous study done by Balli et al 2015, who found that clinical parameters and sclerostin levels were significantly higher in chronic periodontitis patients compared to healthy which subsequently decreased after non-surgical periodontal therapy[20]. Further, inter group comparison of sclerostin levels showed a statistically significant difference at all time intervals ($p < 0.001$) (Table 3). The higher reduction of sclerostin levels in the test group compared to control group could be due to added effect of LLLT biostimulation on osteoblasts thereby leading to increased cell proliferation. LLLT irradiation induces enhanced osteoblast proliferation, intracellular metabolic changes resulting in faster cell division, proliferation and migration of fibroblasts[40, 42, 60, 61]. Literature evidence shows that sclerostin promotes osteoclast formation leading to bone resorption by a RANKL dependent manner and also regulates the PHEX / MEPE axis leading to inhibition of bone formation[15]. From the above-mentioned studies, it is evident that LLLT exerts a negative effect on sclerostin levels, which in turn favours bone formation as observed in the present study.

Radiographic parameters such as defect depth showed a highly significant reduction in both groups at baseline to 3 and 6 months. However, only in the test group at 3 to 6 months, there was a statistically significant reduction (Table 1, 2) this could be attributed to the biostimulatory effects of LLLT leading to progressive improvement in bone remodelling [32, 36, 39, 60, 34]. The positive aspect of the present study was a significant gain in defect height from baseline to 3 months and 3 to 6 months in test group suggesting the role of LLLT in inducing progressive bone formation. This was attained with a single laser irradiation. Moreover, in a previous study there was no further new bone formation at 3 to 6 months in spite of multiple LLLT irradiations and additional use of bioactive glass[50].

In present study the correlation between clinical parameters and sclerostin levels in both groups at various time intervals showed a statistically significant positive correlation (Table 4, 5). These results are in accordance with previous studies done by Balli et al 2015 and Napimoga MH et al 2014[20, 62]. Sclerostin levels were correlated with defect depth in test group at various intervals, showed a statistically significant positive correlation, whereas in control group there was no significant correlation.

The correlation between clinical parameters to defect depth showed a statistically significant positive correlation in test group which is due to the added effects of LLLT biostimulation in intrabony defect site, when compared to control group (Table 4, 5). When clinical parameters were correlated to gain in defect height in test group showed a strong negative correlation. This could be attributed to positive benefits of LLLT in intrabony defect regeneration.

The results of the present study proposes that LLLT biostimulation as an adjunct to OFD has a key role in achieving intrabony defect fill. These findings corroborate with results shown in previous studies. Additionally, the present study also evaluated the effect of LLLT on sclerostin biomarker levels. To the author's knowledge, there is no literature evidence to compare the above data, as the current study is the first of its kind.

However, the present study has a few shortcomings: Discrepancy in baseline PPD and CAL values between test and control groups, single irradiation: Few studies have adopted multiple irradiation strategies during the initial phases of wound healing which showed improved clinical outcomes and short follow-up period.

SUMMARY AND CONCLUSION:

In the present study, LLLT as an adjunct to intrabony defect regeneration along with sclerostin biomarker was evaluated for the first time. There was a significant reduction in postoperative clinical and radiographic parameters and following use of LLLT. Furthermore, LLLT had added effect on reducing sclerostin biomarker levels by its biostimulatory effects on osteoblasts thereby favouring bone formation. The clinical outcomes of the present study could be enhanced by increasing the sample size and longer follow up with multiple irradiations. Further human clinical trials utilizing LLLT on sclerostin levels are warranted. Thus within the limitations of current study it can be concluded that LLLT biostimulation as an adjunct to open flap debridement in surgical management of intrabony defect regeneration shows a beneficial effect as evidenced by improved radiographic bone fill.

No conflicts of interest

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