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CLINICAL AND BIOCHEMICAL EVALUATION OF EFFICACY OF 1% METFORMIN GEL AS AN ADJUNCT TO SRP IN CHRONIC PERIODONTITIS- A SPLIT MOUTH PLACEBO CONTROLLED STUDY.

Dental Science

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ABSTRACT

Carboxy terminal telopeptide of type 1 collagen (ICTP) is a 9KD fragment of Type 1 collagen, which is released into the extracellular fluid during the resorption of mature bone collagen, its concentration in serum has been reported to correlate with bone resorption. Metformin a biguanide which is been widely used as antidiabetic agent has also shown anti osteoclastic effect. Aim: This research was undertaken to asses clinical and biochemical evaluation of efficacy of 1% metformin gel as an adjunct to SRP in chronic periodontitis.

Methodology: 15 patients who are diagnosed with chronic periodontitis were included in the study. The clinical parameters like PI, MGI, BOP, PPD, CAL and GCF concentration of ICTP were evaluated at baseline and 3 months. The patients were subjected for full mouth SRP followed by 1% MF gel application subgingivally.

Results: The findings suggest that short-term nonsurgical therapy and LDD of 1% Metformin resulted in an improvement in clinical signs of inflammation, along with subsequent decrease in GCF ICTP concentration. Reduction in GCF ICTP concentration was much better when there was multiple applications of Metformin Gel.

Conclusion: 1% Metformin gel as an adjunct to SRP shows to decrease the PDL tissue destruction indicating an osteomodulatory effect.

KEYWORDS

Periodontitis, Metformin, ICTP, Osteoclastogenesis, GCF

INTRODUCTION

Periodontal disease is a chronic inflammatory disease characterized by inflamed gingiva, bleeding on probing, attachment loss and resorption of alveolar bone. In the past decade, periodontal disease has been recognized as not merely a local infectious disease but as a chronic, subclinical, inflammatory disease of the host.

As early diagnosis can lead to early intervention and prevention of advancing diseases, the detection of oral fluid based biomarkers and their concentrations can be used to develop a test for early clinical diagnosis which could provide early warning of disease and its pathogenesis in advance of clinical and radiographic evidences. Gingival crevicular fluid(GCF) is a rich pool of proteins and molecules that reflect aspects of oral and systemic health.[1] Many studies have concluded that oral fluid based biomarkers of alveolar bone loss were present in elevated levels in GCF of patients with periodontal diseases.[2,3] Hence, the GCF levels of biomarkers could be of clinical utility as a screening tool for periodontal disease and also to evaluate the healing of periodontal tissues.

Pyridinoline cross linked carboxy terminal telopeptide of type 1 collagen(ICTP)is a 9KD fragment of Type 1 collagen, which is released into the extracellular fluid during the resorption of mature bone collagen, and its concentration in serum has recently been reported to correlate with histomorphometric measurements of bone resorption in a group of patients with various metabolic bone diseases.[4] Many studies have shown that ICTP levels in GCF differed significantly between healthy, gingivitis and periodontitis subjects.[5]

Metformin a biguanide which has been widely used as antidiabetic agent for the treatment of type 2 diabetes mellitus (DM). Metformin is one of the insulin sensitizing agents most commonly used for the management of different conditions associated with insulin resistance, such as Type2 DM, metabolic syndrome, polycystic ovary syndrome etc. Recent studies have shown that the local use of Metformin drug can inhibit the osteoclastogenesis and bone destruction.[6] Therefore this study is undertaken to evaluate the efficacy of 1% Metformin gel in controlling alveolar bone resorption by testing the levels of ICTP in GCF before and after treatment in chronic periodontitis patients.

METHODOLOGY

The study consisted of 15 patients, (9 males and 6 females) in the age group of 34-64 years visiting the Outpatient Department of Periodontics, Coorg Institute of Dental Sciences, Virajpet, Kodagu, Karnataka, India, who met the inclusion criteria were enrolled in the study.

All participants were explained the need and objectives of the study. Only those subjects who gave consent for the study were included in the study. Upon screening and selecting the patients, the following clinical examinations were considered at base line, and 3rd months on the selected quadrants with deepest Probing pocket depth. Plaque Index [Sillness and Loe (1964)], Sulcus Bleeding Index [Muhlemann & Son (1971)], Bleeding on Probing(BOP), Probing pocket depth (PPD) and Clinical Attachment Level (CAL) (standardized using an acrylic stent and measured by using a UNC-15 probe) were taken at baseline which was repeated only at 3 months post-operatively.

The following criteria for patient selection were used:

INCLUSION CRITERIA

- Patients diagnosed with chronic periodontitis.
- Patients aged 34 to 64years.
- Patients who were systemically healthy.
- Atleast one site with Probing pocket depth (PPD) ≥5mm in 3 quadrants.
- Patients who can follow the instructions and maintain a good oral hygiene.

EXCLUSION CRITERIA

- Patients known /suspected allergy to metformin or any other

biguanide group drugs.
- Patients on systemic metformin or other oral antidiabetic therapy.
- Patient having received any form of Periodontal therapy (PDL) three months before the study.
- Patients suffering from kidney diseases.
- Patients who use tobacco in any form.
- Alcoholism.
- Immunocompromised patients.
- Pregnant or lactating females.

In each of selected patients, 3 quadrants with maximum number of sites with deepest Probing pocket depth were determined and randomly allotted into one of the 3 groups.Then all the subjects underwent full mouth SRP by single examiner, followed by each selected quadrants were subjected for Periodontal intervention as follows;

- Group 1 – SRP + placebo gel on 1st and 30th day.
- Group 2- SRP+ test gel(1%MF Gel) on 1st day and placebo gel on 30th day.
- Group 3-SRP+Test gel(1%MF Gel) on 1st and 30th day.

After 3 months, again GCF were collected from the same sites and all clinical parameters were repeated.

Formulation and Pharmacokinetics of MF Gel

The method of preparation of carbopol gel involved slow addition of polymer to the distilled water. Required quantity of carbopol 934P (1%) was slowly added into warm distilled water and stirred slowly until homogenous mixture was obtained. This enables each flake to become hydrated on the surface and increases the rate of solubilisation, whereas rapid addition of all the carbopol 934P to the distilled water results in formation of large lumps requiring many hours to dissolve. This mixture was then allowed to swell (24 hours) to form a homogeneous polymeric gel. The polymeric dispersions were stirred in a magnetic stirrer for 20 min to which benzalkonium chloride (0.01%) solution was added as preservative. The Metformin hydrochloride (1%) solution in small quantity of distilled water was incorporated drop by drop to the prepared polymeric dispersion. The pH and viscosity of the gel were adjusted by addition of triethanolamine. The prepared formulation was packed in autoclaved glass vials and sealed. The Metformin will be released in a sustained manner from the gels. About 97.66% will be released at the end of 8 hours. Biological Half life of Metformin (Plasma) is 4.8 to 7 hours. This half life will not change with the formulation (i.e. gel)

Local Drug Delivery

- Placebo and test gel were introduced accordingly into the selected pockets by means of a syringe with a blunt canula. Proper Isolation care was taken to prevent contamination with Saliva and Blood. The canula was carefully inserted into the periodontal pocket and the placebo and test gel was applied in the test sites in a gentle probing manner, attempting to fill the full extent of the pocket. The gel was applied upto the gingival margin and the excess gel was removed with a sterile gauze and then allowed to set for 5 min without contamination.
- The patients were not prescribed any antibiotics and/or anti-inflammatory agents after treatment.
- The patients were instructed to refrain from chewing hard or sticky food for 1 week.
- The patients were also instructed not to use any other plaque control methods other than normal brushing and rinsing with plain water.
- All patients were recalled for follow-up at regular intervals and examined for any in adherent reactions in the oral cavity.
- Then all the patients were recalled on 30th day of the study.The selected quadrants were cleaned with wet gauge to remove the supragingival plaque if any before reintroducing placebo or test gel into the pockets accordingly.

METHOD OF GCF COLLECTON FOR ESTIMATION OF ICTP

The subjects were asked to rinse the oral cavity vigorously with water to cleanse the teeth of loosely adherent debris. Sites with deepest probing depth were choosed for GCF collection. The selected tooth surface was cleaned with moist cotton supraginigivally. Samples were obtained by placing a colour coded, calibrated, volumetric, micro capillary pipettes with 1µl range. The micro capillary pipettes were placed extra-crevicularly at the entrance of gingival crevice, and 2µl of GCF sample were collected from each site. Samples contaminated with blood or saliva were discarded. The collected GCF sample was immediately transferred into a collection vial containing 0.5ml of phosphate buffer solution. After that, these samples were freeze dried and stored in cold storage at -70ºC until analysed for ICTP with commercially available ELISA kit.(Bioassay Technology Laboratory, Korain Biotech CO.Ltd)

RESULTS

STATISTICAL ANALYSIS

Data collected by experiments were computerized and analyzed using Statistical Package for the Social Sciences (SPSS Version 17). Since the data were contineous and Quantitative type, parametric tests were used for analysis. Mean and Standard Deviation (SD) were calculated for all the parameters. One way analysis of variance (ANOVA) test was used for multiple group comparisons followed by Tukeys Post hoc for group wise comparisons. p value<0.05 was considered as statistically significant with 95% confidence interval.

Total of 15 patients(9 Male and 6 Female) with an age range between 34 to 55 years were included in this study. The mean age of patients was 44.20±5.91yrs.

When compared within the Group, PI and MGI scores reduced significantly from Baseline to 3 months after Periodontal intervention with a statistically significant difference. (Table 1,2,3)

The Mean Bleeding on probing scores within each group was compared between baseline and 3 months. All the 3 groups showed a statistically significant reduction in mean scores after 3 months of intervention.(Table 1,2,3)

Similarly, Probing pocket depths were recorded on selected teeth and the scores were calculated as mean and S.D in each group at baseline and 3 months. In Group 1 the baseline PPD scores were 5.93±0.593 which reduced to 5.26±0.703at 3 months with statistically significant difference (p=0.001)(Table 1). In Group 2 the baseline PPD scores were 5.80±0.676 which reduced to 4.93±0.703 at 3 months with statistically highly significant difference (p=0.000)(Table 2). In group 3 the baseline PPD scores were 5.80±0.676 which reduced to 4.26±0.883 at 3 months which was found to be statistically highly significant difference (p=0.000) (Table 3).

Same way the Clinical attachment level scores of each group were calculated at baseline and 3 months. In Group 1 the baseline CAL scores were 8.86±0.516 and at 3 months it was reduced to 8.20±0.676 which was statistically significant (p=0.001)(Table 1). In Group 2 the baseline CAL scores were 8.80±0.676 and at 3 months it was reduced to 7.93±0.703 which was highly statistically significant (p=0.000)(Table2). In Group 3 the baseline CAL scores were 8.80±0.676 and at 3 months it was reduced to 7.13±0.990 which was found to be statistically highly significant (p=0.000) (Table 3).

ICTP concentration of each group was compared between baseline and 3 months. In Group 1 the baseline ICTP concentration was 57.04± 3.172 and at 3 months it was reduced to 53.08±2.671 which was statistically highly significant (p=0.00)(Table 1). In Group 2 the baseline ICTP concentration was 57.03±3.224 and at 3 months it was reduced to 47.18±2.538 which was statistically highly significant (p=0.000)(Table2). In Group 3 the baseline ICTP concentration was 57.09± 2.455 and at 3 months it was reduced to 38.58± 2.037 which was found to be statistically highly significant (p=0.000) (Table 3).

When Intergroup Comparison was done at Baseline to check the distribution of samples, the samples were equally distributed with respect to all the parameters among all the 3 groups. When compared the difference in the parameters between the groups, they were statistically not significant with P Value >0.05(Table 4).

Similarly, when Intergroup comparison between 3 groups was done, All the clinical parameters showed significant differences in mean value reduction with P Value <0.05 except in Modified gingival index scores. The Mean ICTP scores at 3 months Post op was 53.08, 47.18 and 38.58 in Groups 1,2 & 3 respectively indicating better reduction in Mean ICTP values in Group 3 compared to Group 1 &2. This differences in ICTP values were statistically highly significant with F-Value 134.99 and P-Value 0.000(Table 5).

When multiple comparisons using Turkeys Post hoc test was carried out, Group 3 showed better reduction in Plaque Index scores compared to Group 1 & 2 with P Value 0.049 & 0.003 respectively indicating 2 times subgingival application of Metformin Gel showed anti plaque effect. In relation to MGI, the difference in reduction of mean values was statistically not significant with P Value >0.005(Table 6).Similarly when compared among the group for BOP scores, the Group 3 showed statistically significant reduction in BOP scores compared to other 2 groups(P value<0,05)(Table6). Group 3 showed statistically significant PPD reduction when compared to Placebo group (Group 1) but when compared with single application of Metformin group (Group 2) the difference was statistically not significant (Table 6). In relation to CAL gain, after 3 months, the Group 3 ie, multiple application of Metformin Gel showed better results compared to single application & Placebo group(P value < 0.05)(Table 6). Similarly the reduction in ICTP levels in GCF was more in Metformin group compared to Placebo group with a difference of 5.892. This reduction in ICTP levels were even better in multiple Metformin applications with a difference in mean of 14.502 at 3 months post op. Again when compared between Group 2& 3, the difference in mean was 8.609 with a statistically significant P value < 0.05.(Table 6)

Discussion

Scaling and root planing (SRP) is one of the most commonly employed procedures for the treatment of periodontal diseases and has been used as the "gold" standard therapy in comparison to other therapeutical procedures. Previous studies have proved that most clinical improvement and microbial changes occurred during the first 3 months post SRP. In particular, mean pocket depth and clinical attachment levels showed a marked improvement 3 months post SRP but also continued to show improvement during the maintenance period.[7] Therefore, the follow up period of 3 months was considered in this study.

Metformin (MF) a biguanide, is a widely used anti-diabetic agent for the treatment of type 2 diabetes mellitus since the late 1950s.[6] MF is one of the insulin-sensitizing agents most commonly used for the management of different conditions associated with insulin resistance, such as type 2 diabetes, metabolic syndrome and polycystic ovary syndrome. Recent in vivo and in vitro studies suggest that metformin reduces receptor activator for nuclear factor-kappa B ligand (RANKL) and stimulates osteoprotegerin expression in osteoblasts, further inhibiting osteoclast differentiation and prevents bone loss in ovariectomized rats.[8] In another study on ligature-induced periodontitis, the MF treatment of rats induced a significant reduction in alveolar bone loss compared to vehicle-treated rats.[9]

An important site for antibacterial/Host modulating drug delivery would seem to be from within the periodontal pocket, where local concentrations of the drug at the disease site can be established and maintained at any desired level for any duration required. By means of controlled local delivery from within the periodontal pocket, a single administration of a few milligrams of an antibacterial agent can maintain therapeutic concentrations within the crevicular fluid for a longer period of time than any other mode of delivery.[10]

Therefore, the present study was undertaken to evaluate the Clinical and Biochemical evaluation of the efficacy of 1% Metformin gel as an adjunct to SRP in chronic periodontitis- a Split mouth placebo controlled study.1% Metformin Gel was considered in the study because the results of the previous study showed significant improvement in clinical and radiographic parameters when used in the intrabony defects in patients diagnosed with chronic periodontitis.[11,12]

The clinical parameters considered were PI, GI, BOP, PPD and CAL. Similarly ICTP levels were also assessed at baseline and 3months in all the 3 groups. ICTP has been shown to be a promising predictor of both future alveolar bone and attachment loss.[5] Furthermore, ICTP was strongly correlated with clinical parameters and putative periodontal pathogens, and demonstrated significant reduction after periodontal therapy.[5] Studies assessing the role of GCF ICTP levels as a diagnostic marker of periodontal disease activity have produced promising results to date. **Palys *et al*,** have evaluated Relationship between C-telopeptide pyridinoline cross-links (ICTP) and putative periodontal pathogens in periodontitis.[5] According to author, Crevicular fluid pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen (lCTP) is predictive for future alveolar bone loss in experimental periodontitis in dogs. The results demonstrated significant differences among disease categories for GCF ICTP levels for healthy (1.1±0.6 pg/site (mean±SEM)) gingivitis (14.8±6.6 pg/site) and periodontitis subjects (30.3±5.7 pg/site) *(p*= 0.0017).[5] ICTP levels related modestly to several clinical parameters. For ICTP analysis, 2µ litre of GCF was collected from deepest probing pocket site from each quadrant and then subjected for ELISA to check the levels of ICTP in nanograms (ng).

All 3 groups showed improvement in clinical parameters from baseline to three months. When multiple comparisons using Turkeys Post hoc test was carried out, 2 times application of 1% Metformin Gel showed better reduction in Plaque Index scores compared to Placebo and Single application with P Value 0.049 & 0.003 respectively, indicating 2 times subgingival application of 1% Metformin gel showed better anti plaque effect .This effect was even reflected in MGI and BOP scores (Table 6). Group 3 showed statistically significant PPD reduction when compared to Placebo group (Group 1) but when compared with single application although there was more reduction in PPD the difference was statistically not significant (Table 6). In relation to CAL gain, after 3 months, the Group 3 ie, multiple application of Metformin Gel showed better results compared to single application & Placebo group (p value < 0.05)(Table 6) which was in accordance with previous study done by **Nishanth *et al*** in which 1% Metformin Gel in biodegradable, controlled release as an adjunct to SRP in smokers with generalized chronic periodontitis patients showed greater decrease in modified sulcular bleeding, pocket depth and more gain in CAL.[13] In this study, the SRP alone had showed significant reduction in ICTP levels after 3 months which is contrary to the previous study by **Al-Shammari *et al*** and **Giannobile *et al***, in which non surgical mechanical therapy did not show significant reduction in ICTP and IL-1 levels.[14] Another study by **Golub *et al*.** found that treatment of chronic periodontitis patients with nonsurgical periodontal therapy and LDD with Minocycline resulted in a 70% reduction in GCF ICTP levels after 1 month, with concomitant with a 30% reduction in collagenase levels.[15]

Similarly, the reduction in ICTP levels in GCF was more in Metformin group compared to Placebo group with a difference of 5.892. This reduction in ICTP levels were even better in multiple Metformin application with a difference in mean of 14.502 at 3 months post operatively. Similarly when compared between Group 2& 3, the difference in mean was 8.609 with a statistically significant p value < 0.05(Table 6). This indicates that 2 times application of 1% Metformin Gel as an adjunct to SRP not only had improvement in clinical parameters, but also helped in reducing the bone degeneration by arresting PDL tissue destruction which was in accordance with the previous study conducted by **Pradeep *et al*[11,12]**, which can be attributed to the direct osteogenic effect of Metformin on osteoblasts as confirmed by **Cortizo *et al*[16]** and **Kanazawa *et al*[17]** in Osteoblast culture study. Similarly **Mai *et al*[8]** in invitro and invivo studies suggested that MF reduces receptor activator for nuclear factor-kB ligand (RANKL) and stimulates osteoprotegerin expression in osteoblasts, further inhibiting osteoclast differentiation and preventing bone loss in ovariectomized rats. In another study on ligature-induced periodontitis, MF treatment of rats induced a significant reduction in alveolar bone loss compared with vehicle-treated rats.[9]

Hence, when 1% Metformin gel was placed in intrabony defects surgically there was a significant defect fill was noticed when assessed clinically and radiographically.[15] Therefore, the results from previous and present study shows the osteomodulatory effect of 1% Metformin gel when used as an adjunct to SRP in chronic periodontitis patients which was confirmed by analyzing the ICTP levels in GCF collected from diseased sites. Further studies with larger sample size and longer duration follow up should be conducted to take this research forward.

CONCLUSION

The following conclusions may be drawn from the present study:

1. ICTP can be a useful GCF biomarker for periodontal disease.
2. Following SRP and Local drug delivery of 1% Metformin as an adjunct in chronic periodontitis patients, there is subsequent decrease in GCF ICTP concentration.
3. Reduction in GCF ICTP concentration was much better when there was multiple applications (2 times) of Metformin Gel.
4. 1% Metformin gel as an adjunct to SRP in chronic periodontitis patients shows to decrease/ arrest the PDL tissue destruction indicating an osteomodulatory effect.

Table 1. Comparison of clinical parameters within Group 1 group at baseline and 3 months

Clinical parameters	Duration	Mean	Std. Deviation	t value	P value	Significance
PLAQUE INDEX	BASELINE	1.37	0.214	8.463	0.000	HS
	3 MONTHS	1.30	0.208			
MODIFIED GINGIVAL INDEX	BASELINE	1.34	0.074	8.751	0.000	HS
	3 MONTHS	1.28	0.062			
BLEEDING ON PROBING	BASELINE	0.78	0.099	8.404	0.000	HS
	3 MONTHS	0.73	0.095			
PROBING POCKET DEPTH	BASELINE	5.93	0.593	4.183	0.001	S
	3 MONTHS	5.26	0.703			
CLINICAL ATTACHMENT LEVEL	BASELINE	8.86	0.516	4.183	0.001	S
	3 MONTHS	8.20	0.676			
ICTP	BASELINE	57.04	3.172	7.323	0.000	HS
	3 MONTHS	53.08	2.671			

P<0.05, S – Significant, HS- Highly Significant

Table 2. Comparison of clinical parameters within Group 2 at baseline and 3 months

Clinical parameters	Duration	Mean	Std. Deviation	t value	P value	Significance
PLAQUE INDEX	BASELINE	1.43	0.156	14.642	0.000	HS
	3 MONTHS	1.36	0.164			
MODIFIED GINGIVAL INDEX	BASELINE	1.37	0.085	9.997	0.000	HS
	3 MONTHS	1.27	0.073			
BLEEDING ON PROBING	BASELINE	0.78	0.040	10.058	0.000	HS
	3 MONTHS	0.71	0.024			
PROBING POCKET DEPTH	BASELINE	5.80	0.676	6.500	0.000	HS
	3 MONTHS	4.93	0.703			
CLINICAL ATTACHMENT LEVEL	BASELINE	8.80	0.676	6.500	0.000	HS
	3 MONTHS	7.93	0.703			
ICTP	BASELINE	57.03	3.224	16.113	0.000	HS
	3 MONTHS	47.18	2.538			

P<0.05, S – Significant, HS- Highly Significant

Table 3. Comparison of clinical parameters within Group 3 at baseline and 3 months

Clinical parameters	Duration	Mean	Std. Deviation	t value	P value	Significance
PLAQUE INDEX	BASELINE	1.41	0.122	12.717	0.000	HS
	3 MONTHS	1.15	0.093			
MODIFIED GINGIVAL INDEX	BASELINE	1.34	0.073	17.441	0.000	HS
	3 MONTHS	1.22	0.090			
BLEEDING ON PROBING	BASELINE	0.77	0.042	14.626	0.000	HS
	3 MONTHS	0.65	0.051			
PROBING POCKET DEPTH	BASELINE	5.80	0.676	11.500	0.000	HS
	3 MONTHS	4.26	0.883			
CLINICAL ATTACHMENT LEVEL	BASELINE	8.80	0.676	13.229	0.000	HS
	3 MONTHS	7.13	0.990			
ICTP	BASELINE	57.09	2.455	28.151	0.000	HS
	3 MONTHS	38.58	2.037			

P<0.05, S – Significant, HS- Highly Significant

Table 4. Comparison of clinical parameters between Group 1, Group 2 and Group 3 at baseline

	Groups	Mean	Std. Deviat	F value	P value Significance
PLAQUE INDEX	Group 1	1.37	0.214	0.573	0.568 NS
	Group 2	1.43	0.156		
	Group 3	1.41	0.122		
MODIFIED GINGIVAL INDEX	Group 1	1.34	0.074	0.933	0.401 NS
	Group 2	1.37	0.085		
	Group 3	1.34	0.073		
BLEEDING ON PROBING	Group 1	0.78	0.099	0.145	0.865 NS
	Group 2	0.078	0.040		
	Group 3	0.77	0.042		
PROBING POCKET DEPTH	Group 1	5.93	0.593	0.211	0.811 NS
	Group 2	5.80	0.676		
	Group 3	5.80	0.676		
CLINICAL ATTACHMENT LEVEL	Group 1	8.86	0.516	0.056	0.945 NS
	Group 2	8.80	0.676		
	Group 3	8.80	0.676		
ICTP	Group 1	57.04	3.172	0.001	0.999 NS
	Group 2	57.03	3.224		
	Group 3	57.09	2.455		

***ANOVA test S- Significant, NS- Not Significant, HS- Highly Significant**

Table 5. Comparison of clinical parameters between Group 1, Group 2 and Group 3 at 3 months

	Groups	Mean	std. deviation	F value	P value Significance
PLAQUE INDEX	Group 1	1.30	0.208	6.474	0.004 S
	Group 2	1.36	0.164		
	Group 3	1.15	0.093		
MODIFIED GINGIVAL INDEX	Group 1	1.28	0.062	3.058	0.058 NS
	Group 2	1.27	0.073		
	Group 3	1.22	0.090		
BLEEDING ON PROBING	Group 1	0.73	0.095	6.398	0.004 S
	Group 2	0.71	0.024		
	Group 3	0.65	0.051		
PROBING POCKET DEPTH	Group 1	5.26	0.703	6.586	0.003 S
	Group 2	4.93	0.703		
	Group 3	4.26	0.883		
CLINICAL ATTACHMENT LEVEL	Group 1	8.20	0.676	7.172	0.002 S
	Group 2	7.93	0.703		
	Group 3	7.13	0.990		
ICTP	Group 1	53.08	2.671	134.99	0.000 HS
	Group 2	47.18	2.538		
	Group 3	38.58	2.037		

***ANOVA test S- Significant, NS- Not Significant, HS- Highly Significant**

Table 6. Multiple group comparisons using tukeys post hoc test

Clinical parameter	Group	Compared with	Mean Difference	Sig.
PLAQUE INDEX	Group 1	Group 2	0.064	0.533
		Group 3	**0.144***	**0.049**
	Group 2	Group 3	**0.208***	**0.003**
MODIFIED GINGIVAL INDEX	Group 1	Group 2	0.004	0.985
		Group 3	0.062	0.079
	Group 2	Group 3	0.057	0.112
BLEEDING ON PROBING	Group 1	Group 2	0.019	0.692
		Group 3	**0.080***	**0.004**
	Group 2	Group 3	**0.061***	**0.033**
PROBING POCKET DEPTH	Group 1	Group 2	0.333	0.467
		Group 3	**1.000***	**0.003**
	Group 2	Group 3	0.666	0.056
CLINICAL ATTACHMENT LEVEL	Group 1	Group 2	0.266	0.637
		Group 3	**1.066***	**0.002**
	Group 2	Group 3	**0.800***	**0.025**
ICTP	Group 1	Group 2	**5.892***	**0.000**
		Group 3	**14.502***	**0.000**
	Group 2	Group 3	**8.609***	**0.000**

***. The mean difference is significant at the 0.05 level.**

REFERENCES

1. Guthmiller JM, Brogden KA. Influence of smoking on GCF cytokines in severe chronic periodontitis. J clin periodontol 2011;38:219-28
2. Fine DH,Markowitz K,Furgang D,Nasri C. Macrophage inflammatory protein 1 alpha;A Salivary biomarker of bone loss in a longitudinal cohort study of children at risk for Aggressive periodontal disease. J periodontol 2009;80:106-13.

3. Sexton WM ,Lin Y. Salivary Biomarkers of periodontal disease in response to treatment. J clin periodontol 2011; 38:434-41.
4. Hassager C,Jensen LT,Pedenphant J,Thomsen K,Christiansen C .The carboxy- terminal pyridinoline cross-linked telopeptide of type 1 collagen in serum as a marker of bone resorption:the effect of nandrolone decanoate and hormone replacement therapy. Calcif Tissue Int 1994;54:30-3.
5. Palys,M.D, A.D Haffajee, S.S Socransky& W.V Giannobile. Relationship between ICTP and putative periodontal pathogens in periodontitis. J Clin Periodontol.1998; 25(11 pt 1) :865-71.
6. Gao Y, Li Y, Xue J, Jia Y, Hu J. Effect of the anti-diabetic drug metformin on bone mass in ovariectomized rats. Eur J Pharmacol. 2010 Jun 10;635(1-3):231-6
7. Cugini M A, Haffajee A D, Smith C, Kent Jr. RL, Socransky S S: The effect of scaling and root planing on the clinical and microbiological parameters of periodontal diseases: 12-month results. J Clin Periodontol 2000;27:30-6.
8. Mai QG, Zhang ZM, Xu S. Metformin stimulates osteoprotegerin and reduces RANKL expression in osteoblasts and ovariectomized rats. J Cell Biochem. 2011; 112: 2902-2909.
9. Bak EJ, Park HG, Kim. The effect of metformin on alveolar bone in ligature induced periodontitis in rats: a pilot study. J Periodontol. 2010; 81: 412-419.
10. J. M. Goodson,S. Offenbacher, D. H. Farrf and P. E. Hogan. Periodontal Disease Treatment by Local Drug Delivery. J. Periodontol. May, 1985;56:265-72
11. Pradeep AR, Rao NS, Naik SB, Kumari M. Efficacy of Varying Concentrations of Subgingivally Delivered Metformin in the Treatment of Chronic Periodontitis: A Randomized Controlled Clinical Trial. J Periodontol 2013; 84:212-220.
12. Pradeep AR, Patnaik K, Nagpal K, Karvekar S, Ramamurthy BL, Naik SB. Efficacy of locally-delivered 1% metformin gel in the treatment of intrabony defects in patients with chronic periodontitis: a randomized, controlled clinical trial. J Investig Clin Dent 2015.
13. Rao NS, Pradeep AR, Kumari M, Naik SB. Locally Delivered 1% Metformin Gel in the Treatment of Smokers with Chronic Periodontits: A Randomized Controlled Clinical Trial. J Periodontol 2013; 84(8):1165-1171.
14. Al-Shammari KF, Giannobile WV, Aldredge WA, Iacono VJ, Eber RM, et al. (2001) Effect of non-surgical periodontal therapy on C-telopeptide pyridinoline cross-links (ICTP) and interleukin-1 levels. J Periodontol 72: 1045-1051
15. Golub LM, Lee HM, Greenwald RA. A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. Inflamm. Res 1997;46: 310–319.
16. Cortizo AM, Sedlinsky C, McCarthy AD, Blanco A, Schurman L. Osteogenic actions of the antidiabetic drug metformin on osteoblasts in culture. Eur J Pharmacol. 2006; 536: 38-46.
17. Kanazawa I, Yamaguchi T, Yano S, Yamauchi M, Sugimoto T. Metformin enhances the differentiation and mineralization of osteoblastic MC3T3-E1 cells via AMP kinase activation as well as eNOS and BMP-2 expression. Biochem Biophys Res Commun. 2008; 375(3):414-419.