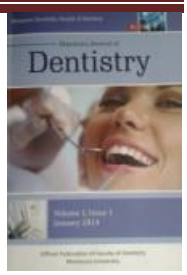
" Therapeutic Efficacy of Locally Delivered Metformin Gel Versus Clindamycin Gel in Chronic Periodontal Diseases"



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Abstract:
Background: Periodontitis is a disease with multifactorial etiology and bacteria are one among these etiologic agents. Although a number of surgical and pharmacological options are available for the management of periodontitis, it still affects a large proportion of population. Recently, Metformine which is an oral anti-hyperglycemic drug has shown to increase osteoblastic proliferation .On the other hand, Clindamycin, a lincosamide antimicrobial agent which in addition to its direct antibacterial effect on ribosomal units, it has a number of unique pharmacological features that enhance its clinical efficacy and reaches high concentrations in saliva, gingival cervical fluid and bone. Patients with periodontal diseases have high levels of GCF alkaline phosphatase enzyme (ALP), so it can be used as potential marker for disease activity.
Aim: of our study was to evaluate the effect of 1% metformin gel versus 2% clindamycin gel as a local drug delivery in treatment of chronic periodontitis patients, and assessment of alkaline phosphatase enzyme levels in the gingival crevicular fluid of such patients.
Subjects and methods: This study was conducted on thirty patients, who were diagnosed as having chronic periodontitis. In addition to five healthy subjects were chosen as control subjects (group IV). Patients were divided into 3 groups: I, II, and III. 1% metformin gel was applied to group I, 2% clindamycin gel was applied to patients in group II, while scaling and root planning only for group III, SRP and local drug delivery was applied for patients once weekly for six weeks. Clinical periodontal parameters (periodontal pocket depth, clinical attachment loss and bleeding on probing, plaque index and gingival index) were recorded for all groups at baseline and six weeks after treatment for groupI, II and groupIII. GCF samples were taken at baseline for all groups and six weeks after treatment for group I, II and III. The samples were analyzed photometricaly .
Results: All periodontal parameters (PI, GI, PBI, PPD, CAL) and ALP levels were reduced significantly after six weeks.
Conclusion: 1% percent MF was found to significantly improve clinical and parameters and decrease AlP levels in patients with chronic periodontitis, also it can be concluded that the utilization of 2% clindamycin gel in combination with SRP enhances the efficacy of nonsurgical periodontal therapy in reducing pocket depth and improving attachment levels in chronic periodontitis subjects and dereasing ALP levels, so both drugs are very useful as an adjunct treatment moreover, clindamycin gel is more effective in treatment of chronic periodontitis.

Introduction

Periodontal disease is a disease, or more likely a number of diseases of the periodontal tissues that results in attachment loss and destruction of the alveolar bone surrounding teeth. Most commonly the natural history of periodontal disease, in some but not all patients, results in tooth loss finally [1].

Treatment of periodontal disease is aiming at the removal of pathogenic bacteria, correction of reversible risk factors, and then the prevention of recolonization in order to prevent disease recurrence. The standard nonsurgical treatment for periodontal disease is scaling and root planing (SRP). [2] Adjunctive systemic and/or local antimicrobial treatment has been found to positively impact periodontal therapy outcomes. [3]

While a great focus has been on managing the inflammation in the gingival tissues, advances in our understanding of bone metabolism are opening up new avenues of understanding pathological bone loss in periodontitis. This knowledge, together with the development of novel drugs that can inhibit bone loss/destruction, provide us with opportunities to target not only soft tissue inflammation, but also the destructive bone loss in periodontitis [4].

Metformin (MF) is a biguanide that is one of the most common oral hypoglycemic drugs used in type 2 diabetes mellitus [5]. Numerous studies have reported the effects of this agent on bone turnover. The action of MF on the development of osteoblast-like cell lines was investigated for the first time by Cortizo et al. [6], who found a direct osteogenic effect of MF on osteoblasts in culture.

Clindamycin is a derivate of lincomycin that is more active and has fewer side-effects than the parent drug [7]. It is an antimicrobial agent that has been in use worldwide for more than 3 decades, has been consistently effective in the treatment of infections involving a wide spectrum of facultative and strictly anaerobic bacteria [8].

With regard to strict anaerobes, clindamycin has activity against a range of gram-negative species, including Porphyromonas species, Prevotella species, Bacteroides fragilis group, Veillonella species, and Fusobacterium species, including b-lactamasee producing strains. [9]

In the periodontium, alkaline phosphatase is a part of the normal turnover of periodontal ligament, root cementum formation and maintenance, and bone homeostasis. It is

associated with the calcification process and an elevated ALP level commensurate with active bone remodelling.

ALP and periodontal disease in an experimental gingivitis model showed a significant correlation between ALP and pocket depth and between ALP and inflammation. As a predictive indicator for future periodontal breakdown, ALP therefore might serve as a marker in periodontal treatment planning and monitoring. [10]

Subjects and Methods:

Patient selection: This study was carried on thirty patients and five control subjects were included as control group in our study. The subjects were selected from those attending Oral Medicine and Periodontology Department, Faculty of Dentistry, Mansoura University. They were 11 males and 24 females. Their ages ranged from 30 to 60 years. The selected subjects were divided into four groups .

Study groups: Group I: Ten patients having chronic periodontitis were treated with scaling and root planning in addition to 1% Metformin gel once weekly as local drug delivery system.

Group II: Ten patients having chronic periodontitis were treated with scaling and root planning in addition to 2% Clindamycin gel once weekly as local drug delivery system.

Group III: Ten patients having chronic periodontitis were treated with scaling and root planning only once weekly.

Group IV (control group): Five healthy subjects without any systemic diseases with healthy gingiva were selected as control group.

Inclusion criteria: 1) Chronic periodontitis patients with pocket depth more than 4 mm depth. 2) Age ranged from 30-60 years old.

Exclusion criteria: 1) Patient with known systemic disease such as diabetes or blood disease. 2) Patients suspected to be allergic to the MF/biguanide group of drugs. 3) Patients suspected to be allergic to clindamycin/lincosamide group of drugs. 4) Patients taken antimicrobial therapy, non-steroidal anti-inflammatory drugs, corticosteroid therapy for the last 6 months. 5) Patients undergone periodontal therapy in the preceding 6 months. 6) Patients with aggressive periodontitis. 7) Smokers patients. 8) Immunocompomised patients. 9) Pregnant and lactating females.

Clinical Assessment: Proper case history was taken from each patient, also the onset and duration of the patient's periodontal status was reported as well as any past dental treatment.Patients were exposed to thorough clinical oral and extraoral examination.

Periodontal Assessment:

Periodontal indices:

- Plaque index [11]
- Gingival index.[12]
- Papillary bleeding index (PBI). [13]
- Probing pocket depth: Probing will be performed with a calibrated periodontal probe. [14]
- Clinical attachment level (CAL). [15]

Periodontal treatment: Collection of GCF samples from all subjects in all groups including healthy control group before starting the treatment and after six weeks of treatment for the study groups, Phase I therapy including scaling and root planning for all patients in groups I, II and III, Injection of 1% Metformin gel in the periodontal pocket of all patients in group I, repeating injection once weekly for six weeks, and then collecting GCF sample at the end of the sixth week, Injection of 2% Clindamycin gel in the periodontal pocket of all patients in group II, repeating injection once weekly for six weeks, and then collecting GCF sample at the end of the sixth week, complete scaling and root planning for all patients in group III once weekly and maintaining oral hygiene measures once weekly for six weeks, then collecting GCF samples at the end of the sixth week.

Methods of application of local drug delivery system:

After debridement, a single dose of the investigational product which is 1% Metformin gel for group I and 2% Clindamycin gel for group II was applied into the periodontal pockets with a syringe and a blunt cannula. The cannula was inserted into the base of the periodontal pocket, and the gel was applied until excess gel flowed out of the pocket. [16]

Sample Collection and storage of gingival crevicular fluid (GCF):

Samples were collected from all subjects in the four groups at base line, and six weeks after therapy in groups I, II and III. Each periodontal site included in the study was isolated with cotton rolls. Before GCF collection, any supra-gingival plaque was removed with cotton pellets, [17]. The GCF was collected using #30 standardized sterile paper strips§ [18] inserted 1 mm into the gingival crevice and left in situ for 30 seconds. Care was taken to avoid mechanical injury, and samples with blood were discarded. Immediately after collection, the paper points were transferred to Eppendorf tubes containing phopsphate buffer 7% and were analyzed on the same day.

Gel formulation: This method was carried out for the preparation of both 2% Clindamycin gel and 1% Metformin gel as follows: An accurately weighted polymer of carboxy methyl cellulose sodium salt was dispersed in distilled water in which the preservative mixture (0.18% w/w methyl paraben and 0.02% w/w) was previously dissolved. The dispersion was mixed until a clear transparent gel free from air bubbles was obtained. The required weight of drug was dissolved in propylene glycol and distilled water and added to the gel with stirring until homogenous distribution. The weight of gel was adjusted to 100 gm, and then packaged in clean, dry and sterile glass containers, kept refrigerated at temperature 3 °C until used.

Assessment of Alkaline phosphatase assay in GCF:

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is hydrolyzed by phosphatases to form phosphate and p-nitrophenol which is released and its presence is proportional to ALP activity and can be measured photometrically.(19)

Statistical analysis: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.

Results:

This study revealed that there was a statistically significant difference regarding clinical parameters (GI, PI, PBI, PPD, CAL) before and after treatment in all study groups ($p \leq 0.001$). with greatest improvement in group II treated with clindamycin gel followed by group I treated with metformin gel, then the least improvement was for group III treated with SRP alone these results are summarized in **table (1).**

Table (1): showing clinical periodontal parameters after treatment between studied groups.

After treatment	Metformin Gel Group I	Clindamycin Gel Group II	SRP Group III	Control Group IV	F	P
Gingival index Mean±SD	0.67 ± 0.22^{AB}	0.53 ± 0.24^{A}	1.43 ± 0.20	0.89 ± 0.37^{B}	25.55	<0.001* p1=0.11 p2=0.01* p3<0.001* p4=0.21 p5<0.001* p6<0.001*
Plaque index Mean±SD	0.80 ± 0.27^{A}	0.48 ± 0.24^{B}	1.50 ± 0.21	0.54 ± 0.35^{AB}	30.8	<0.001* p1=0.07 p2=0.66 p3<0.001* p4=0.008* p5<0.001* p6<0.001*
PBI Mean±SD	0.141 ± 0.14^{B}	0.08 ± 0.07^{AB}	0.49 ± 0.11	0 ± 0.0^{A}	35.8	<0.001* p1=0.02* p2=0.21 p3<0.001* p4=0.19 p5<0.001* p6<0.001*
PD Mean±SD	2.03 ± 0.21	1.70 ± 0.35^{A}	2.36 ± 0.19	1.39 ± 0.39^{A}	16.18	<0.001* p1<0.001* p2=0.06 p3<0.001* p4=0.015* p5=0.013* p6<0.001*
CAL Mean±SD	1.99 ± 0.48^{AB}	1.51 ± 0.42^{A}	2.23 ± 0.73^{B}	0 ± 0	22.32	<0.001* p1<0.001* p2<0.001* p3<0.001* p4=0.051 p5=0.31 p6=0.004*

F: One Way ANOVA test p: probability *statistically significant ($p<0.05$)Similar letters in same row denote non significant difference between groups.
p1:difference between control& Metformin groups, p2: difference between control & Clindamycin group & p3: difference between control and SRP group , p4: difference between Metformin & Clindamycin group , p5: difference between Metformin and SRP groups , p6: difference between Clindamycin and SRP groups.

Regarding alkaline phosphatase enzyme levels, there was astatistically significant decrease in the study groups before and after treatment. However ALP showed higher significant difference comparing group II (clindamycin gel) with group I (metformin gel) (P=0.036), and with group III (SRP) with le least value was for group III. These results are summarized in **table (2)**.

Table (2): Comparison of mean alkaline phosphatase level after treatment between studied groups

ALP after treatment	Metformin Gel Group I	Clindamycin Gel Group II	SRP Group III	Control Group IV	F	P
ALP Mean±SD	124.29±16.3^{B}	94.63±26.4^{A}	136.93±45.6^{B}	86.32±14.6 ^{A}	5.1	0.006* p1=0.029* p2=0.62 p3=0.005* P4=0.036* P5=0.36 P6=0.004*

F: One Way ANOVA test p:probability *statistically significant (p<0.05)Similar letters in same row denote non-significant difference between groups
p1: difference between control& Metformin groups, p2: difference between control & Clindamycin group & p3: difference between control and SRP group, p4: difference between Metformin & Clindamycin group, p5: difference between Metformin and SRP groups, p6: difference between Clindamycin and SRP groups.

Discussion:

Periodontal diseases range from the relatively benign form, such as gingivitis, to chronic and aggressive forms of periodontal disease, all of which not only threaten the dentition, but might also be a threat to general health. (20)
Treatment of periodontal disease is aiming at the removal of pathogenic bacteria, correction of reversible risk factors, and then the prevention of recolonization in order to prevent disease recurrence(21).
At the end of our study, the study group I treated with 1% metformin gel showed a greater reduction in all clinical parameters including PD, and gain in CAL, a decrease in the PBI, GI and PI before and after treatment, this improvement could be attributed to bone-protective effects of metformin by increasing osteoblastic proliferation and increasing formation of type I collagen. (22)
Also there was a statistically significant difference between clinical outcomes in group I more than in group III (SRP) alone. These results go along with a study performed by **Iqra Mushtaq et al**., 2018 (23) that was designed to compare effect of 1% metformin gel as an adjunctive to scaling and root planning to SRP alone, this study showed greater significant reduction in PD and CAL gain in the first group with metformin gel + SRP than the second group with SRP alone.
Shariq Najeeb et al., 2018 in their systematic review and meta-analysis stated that Metformin is an effective medicament in improving the outcomes of surgical and non-surgical periodontal therapy. (24)
Regarding Clindamycin gel, in our study there was statistically significant reduction in all clinical parameters including PI, GI, PD and CAL gain in group II treated with 2% clindamycin gel. This could be attributed to the mechanism of the drug works primarily by binding to the 50s ribosomal subunit of bacteria. This agent disrupts protein synthesis by interfering with the transpeptidation reaction, which thereby inhibits early chain elongation. (25)
Comparing group II with group III (SRP) alone, inter group analysis showed that there was a significant decrease in all clinical parameters in group II more than group III, these results go along with the study performed by **Ana Pejčić et al.,**2015 (26) that was designed to evaluate the therapeutic Efficacy of 2% Clindamycin gel as an Adjunct to Scaling and Root Planning in Chronic Periodontal Disease who showed that clindamycin gel was a good adjunct to SRP therapy at the examined depth of periodontal pocket. Also, they confirmed that mechanical treatment combined with clindamycin gel application was more effective and clinical improvements were predominant. (26)
Regarding ALP enzyme levels, in our study there was a statistically significant decrease in its levels after treatment, this is attributed to arrest of inflammatory process and disease progression whether by SRP combined with LDD as seen in group I & II or SRP alone in group III. This decrease is due to decrease in number of crevicular polymorphneuclear leucocytes (CPLMNS) which play a vital role in pathogenesis of periodontal lesions by migrating into the sulcus.[27] The sources of ALP are polymorphonuclear leukocytes (PMNL),[14] bacteria within the dental plaque[28] and osteoblasts and fibroblast cells.[29]
In group I treated with metformin gel, there was a statistically significant decrease in ALP levels after treatment, this could be due to the inhibitory effect of metformine on RANKL/ OPG ratio. Hence decreasing RANKL inhibit osteoclastic activity and tissue destruction.(30)
While in group II treated with clindamycin gel, our study showed significant decrease in ALP levels may be attributed to the anti-inflammatory properties of clindamycin that leads to further decrease in PMNLS thus decreasing ALP level, also its antimicrobial properties against strict anaerobes and a wide range of gram-negative species, including Porphyromonas species, Prevotella species e.g. p.intermedia and p. gingivalis which are involved in the pathogenesis of chronic are known to have high ALP activity.[31] this leads to more decrease in ALP level as a result of using Clindamycin gel.

In our study the decrease of ALP levels in group III after treatment with SRP alone was due to decrease in inflammation and PMNLS as a result of mechanical debridement and plaque control, this study goes along with **Kunjappu, et al.,** 2012 in their in vivo study to assess the

alkaline phosphatase level in gingival crevicular fluid, as a biomarker to evaluate the effect of scaling and root planing on chronic periodontitis, their study showed that there was statistically significant decrease in ALP levels after scaling and root planning.(32)

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