Clinical and Biochemical Evaluation of the Use of Locally Delivered Metformin as an adjunctive therapy to Non Surgical Periodontal Treatment of Periodontitis Patients

(Randomized Controlled Study)

Protocol Submitted to Department of Oral Medicine,
Periodontology and Oral Diagnosis, Faculty of Dentistry,
Ain Shams University In Partial Fulfillment of the Requirements
for Masters' Degree in Periodontology

Faculty of Dentistry

Ain Shams University

2019

Review of literature

Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus. Its primary features include the loss of periodontal tissue support, manifested through clinical attachment loss (CAL) and radiographically assessed alveolar bone loss, presence of periodontal pocketing (that are a hallmark of the disease and can eventually lead to tooth loss) and gingival bleeding ***(Papapanou et al., 2018).***

The classification of periodontal diseases and conditions in 1999 included gingival diseases including (dental plaque-induced gingival diseases and non-plaque-induced ginigival lesions), chronic periodonitits, aggressive periodonitits, periodonitits as a manifestation of systemic diseases, necrotizing periodontal diseases, abscess of the periodontium, periodontitis with endodontic lesions and developmental or acquired deformities ***(Armitage, 1999).***

The new classification of periodontal and peri-implant diseases and conditions in 2017 classified these diseases into four main categories: First category concerned with periodontal health & gingival diseases either dental biofilm induced gingivitis and non-dental biofilm induced gingivitis. Second category concerned with periodonitits which can be classified into necrotizing periodontitis, periodontitis as a manifestation of systemic disease and periodontitis which involve the forms of the disease previously recognized as “chronic” or “aggressive”. Third category concerned with other conditions affecting the periodontium as systemic diseases, traumatic occlusion, mucogingival deformities, tooth related factors, periodontal abscesses and endodontic-periodontal diseases. Fourth category concerned with peri-implant diseases and conditions as peri-implant health, peri-implant mucositis, peri-implantitis and peri-implant soft & hard tissue deficiencies.

Periodontitis is described in four stages which depend on clinical attachment loss (CAL) ranging from Stage 1: Initial periodontitis (CAL 1-2 mm), Stage 2: Moderate periodontitis (CAL 3-4 mm), Stage 3: Severe periodontitis with potential for additional tooth loss and Stage 4: Severe periodontitis with potential for loss of dentition (CAL ≥5 mm). Grading focuses on assessing risk factors as smoking, systemic considerations as diabetes, and outcomes of non-surgical periodontal therapy. Grade A: Slow rate of progression (no CAL loss over 5 years), Grade B: Moderate rate of progression (CAL loss <2 mm over 5 years) and Grade C: Rapid rate of progression (≥2 mm over 5 years) ***(Caton et al., 2018).***

The pathogenesis of periodontal diseases is mediated by the inflammatory response to bacteria in the dental biofilm. The immune response to infection is regulated by cytokine and chemokine signals. Cytokines and chemokines (chemotactic cytokines) are the messages between cells. Cytokines are low-molecular-weight proteins involved in the initiation and further stages of inflammation, in which they regulate the amplitude and the duration of the response. The genetic regulation leading to the secretion of proinflammatory cytokines from a variety of cells is generally dependent on the activation of nuclear factor kappa-B transcription. Cytokines are produced by resident cells, such as epithelial cells and fibroblasts, and by phagocytes (neutrophils and macrophages) in the acute and early chronic phases of inflammation, and by immune cells (lymphocytes) in established and advanced lesions. After recognition and presentation of microbes to the appropriate cells, cytokines of the innate response, including tumor necrosis factor alpha, interleukin-1beta and interleukin-6, are the first to appear in the periodontal disease pathogenesis pathways. It is suggested that individuals who produce high levels of these mediators in response to the dysbiosis will experience more and severe tissue loss ***(Hanada &Yoshimura, 2002).***

As an adjunctive measurement to aid proper diagnosis, evaluation of levels of various biomarkers is one possible tool. A biomarker is an indicator of a biological situation, which can help to distinguish between health and disease ***(Li & Wang, 2014)***. In periodontology, one of the main challenges is to identify diagnostic and prognostic biomarkers for determination of disease activity and to differentiate between health and disease ***(Buduneli & Kinane, 2011)***.

Sources of biomarkers for periodontal disease include saliva, serum and gingival crevicular fluid ***(Ghallab, 2017)***. GCF is an inflammatory exudate that can be collected at the gingival margin or within the gingival crevice which contains microorganisms, their products, and host derived substances (cytokines, enzymes, immunoglobulins, tissue degradation products, immune system cells) ***(Bostanci, & Belibasakis, 2018).***

Many studies examined bone resorption biomarkers such as RANKL and OPG in GCF or in health and disease. RANKL is a TNF family member protein, in health, its principal sources are osteoblasts, in disease, it is synthesized mostly from T and B lymphocytes ***(Kawai et al., 2006).*** Receptor activator of nuclear factor kappa-beta (RANK) is the receptor of RANKL, located on pre-osteoclasts. RANK-RANKL interaction activates transcription factors of osteoclastogenesis ***(Teitelbaum & Ross, 2003).*** OPG is a soluble glycoprotein, the decoy receptor of RANKL, is synthesized from many different types of cells, Formation of RANKL-OPG complex inhibits

RANK-RANKL interaction via blocking their binding sites ***(Baud'huin et al., 2013)***. Elevated concentrations of RANKL in GCF of periodontitis when compared to healthy areas were demonstrated by ***(Gürlek et al., 2017).***

Lowering bacterial load and inhibiting progression of inflammation are the primary treatment goals that are achieved by mechanical debridement such as scaling and root planing (SRP), a gold standard treatment for periodontitis ***(Socransky & Haffajee, 2000).*** However, SRP shows physical limitations in certain inaccessible areas such as deep periodontal pockets, furcations and interproximal areas of misaligned teeth, which does not allow complete reduction of anaerobic infection and recurrence ***(Cobb, 2005).***

Considering these difficulties, various adjunctive treatments have been investigated to supplement Scaling and Root Planing as local delivery drugs. These include systemic and localized delivery of antibiotics (Clarithromycin gel, minocycline microspheres), chlorohexidine, bisphosphonates, statins & photodynamic therapy to reduce bacterial counts and improve clinical periodontal parameters such as probing depth reduction, gaining clinical attachment level and bone defect (BD) fill in periodontitis ***(Zandbergen et al., 2013), (Akram et al., 2016), (Abduljabbar et al., 2017).***

Metformin a biguanide used in treatment of diabetes mellitus type II as an oral hypoglycemic drug, has shown stimulating effect on osteoblastic lineages leading to osteoblast differentiation and bone formation. MF increased type-I collagen production in both osteoblast-like cell lines and stimulated alkaline phosphate activity in MC3T3E1 osteoblasts ***(Cortizo et al., 2005).*** Since MF benefits on fracture reduction and takes positive action on osteoblasts, it appears possible that this agent may have protective effects on bone loss. Recent in vivo and in vitro studies suggest that metformin reduces receptor activator for nuclear factor-kappa B ligand (RANKL) and stimulates osteoprotegerin (OPG) expression in osteoblasts, further inhibits osteoclast differentiation and prevents bone loss in ovariectomized rats ***(Mai et al., 2011).*** 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 ***(Bak et al., 2010).***

Randomized clinical trials regarding the use of Locally delivered metformin in periodonitits, The 1% MF gel used along with Scaling and Root Planing was found to provide improvement in clinical and radiographical parameters confirmed by Studies done by ***(Rao et al., 2013) & (Pradeep et al., 2017)*** in the form of significant increase in the PD reduction, CAL gain, and improved IBD depth reduction in vertical bone defects. So In this study we will evaluate The 1% MF gel along with SRP was found to provide improvement in clinical and radiological parameters

confirmed by Studies done by **(Rao et al., 2013) & (Pradeep et al., 2017)** in the form of significant increase in the PD reduction, CAL gain, and improved IBD depth reduction in vertical bone defects. So In this study we will evaluate the effect of the locally delivered metformin in the form of clinical and biochemical assessment.

Aim of Study

Primary outcome :

To evaluate the clinical parameters before & after the application of locally delivered metfromin as an adjunctive therapy to non surgical periodontal treatment of Stage III periodontitis.

Secondary outcome :

To measure the RANKL and OPG levels in GCF before & after application of metformin as an adjunctive therapy to non surgical periodontal treatment of Stage III periodontitis.

Subjects and Methods

Study design and Sample size calculation :

In this randomized controlled study, total sample size of 16 patients (8 patient/group) were selected for this study. The sample size was calculated to yield a 95% power in the results of this study based on the results of a previous study (Pradeep et.al., 2017) in which the authors found a significant difference in IBD depth between test and placebo groups (effect size=.2.08 mm, alpha (2 tailed)=.05). The number will be increased to 20 in each group to compensate for dropouts.

Patient Selection :

Forty patients, having periodontitis, will participate in this randomized clinical study. The subjects will be recruited consecutively from the outpatient clinic of Oral Medicine, Periodontology, oral Diagnosis and Radiology Department, Faculty of Dentistry, Ain Shams University The purpose of the study will be explained to all patients and an informed consent will be signed before the conduction of the study. The proposal will be presented to the faculty of Dentistry Ain Shams University Research Ethics committee and have to be approved before starting the research

Inclusion Criteria will be :

1. Patients with Stage III & IV Periodontitis.
2. Age range (30 - 50) years.
3. Good compliance with the plaque control instructions following initial therapy.
4. Availability for follow up and maintenance program.

Exclusion Criteria will be :

1. Smokers
2. Systemic diseases which could influence the outcome of the therapy (According to Cornell Medical Index-Health Questionnaire).
3. Pregnant and lactating females.
4. Vulnerable groups of patients' e.g (prisoners, handicapped patients and decisionally impaired individuals).

For all patients who are suitable for the study the following clinical evaluation parameters will be measured :

a. Plaque index (PI) *(Silness & Löe, 1964)*
b. Gingival index (GI) *(Löe & Silness, 1963)*
c. Probing depth (PD) *(Caton, 1980).*
d. Clinical attachment level (CAL) *(Ramfjord, 1967)*.
e. Standardized Periapical Radiograph

- All evaluations will be done pre operatively (base line) and 6 months post operatively.
- Full mouth assessment will be done and then the deepest areas will be evaluated to receive the local drug.

Grouping Criteria :

The study consists of two groups, each containing 20 patients. Patients will be randomly selected using computer generated randomization (www.randomizer.org) and this will be performed by another individual other than the investigator.

Group I (20 patients) : (Treatment Group)

Supra and sub gingival scaling and root planning will be performed for patients having Stage III Periodonitits. Followed by local application of metformin gel. Oral Hygiene measures will be instructed following treatment and maintenance visits will be given to them.

Group II (20 patients) : (Control Group)

Supra and sub gingival scaling and root planning will be performed to Stage III Periodonitits patients. Followed by local application of placebo gel. Oral Hygiene measures will be instructed following treatment and maintenance visits will be given to them.

Metfromin Preparation :

MF gel will be prepared, briefly all the required ingredients of the formulation will be weighed accurately. Dry gellan gum powder will be dispersed in distilled water maintained at 95°C. The dispersion will be stirred at 95°C for 20 min using a magnetic stirrer to facilitate hydration of gellan gum. The required amount of mannitol will be added to the gellan gum solution with continuous stirring and the temperature will be maintained above 80°C. Weighed amount of MF will be added with stirring. Then sucralose, citric acid, and preservatives (methylparaben, propylparaben) will be added with stirring. Finally, required amount of sodium citrate will be dissolved in 10 ml of distilled water and will be added to the mixture. The mixture will be allowed to cool to room temperature to form gel. (***Mohapatra et al., 2008)***

Steps of the Research :

1. After enrollment, a detailed case history will be recorded.
2. Initial therapy by full mouth supra and subgingival scaling and root planing using ultrasonic scaler and hand instruments will be done on all patients.
3. The patients will be given instructions for self-performed plaque control measures with soft dental brush and interdental cleaning using dental floss or interdental brush.
4. Baseline sampling and the local drug delivery in both study and control groups will be performed next day of scaling to avoid contamination by blood. Samples from the gingival crevicular fluid will be collected from all selected sites using standardized sterile periopaper inserted into the deepest part of each periodontal pocket and left in situ for 30 seconds.
5. The area will be completely dried using oil free air syringe, and then the site will be isolated with cotton rolls to prevent contamination from saliva. The local drug delivery gel will be placed in the periodontal pockets using a dedicated syringe until the gel flowed out from the gingival margin.

Outcomes Assessment :

a) Clinical Assessment

Clinical Assessment will be done using William's graduated probe at baseline and six months (180 days) later.

b) Biochemical Assessment

Biochemical assessment will be done at baseline, and one month after local drug delivery. RANKL & OPG concentrations will be determined using a commercial human RANKL & OPG ELISA Kits. Measurements will be performed according to the manufacturer's instructions.

Statistical Analysis :

- All data will be collected, tabulated and statistically analyzed by appropriate statistical program.

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