Assessment of Wound Healing Using MMP-8 Levels in GCF of Diabetics With Chronic Periodontitis After Diode Laser Assisted Flap Surgery

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ABSTRACT

Background: Inflammatory cytokines like Matrix Metalloproteinases (MMPs) are associated with the destruction observed in periodontal disease. There has been evidence of significant increases in MMP levels in patients with systemic disorders, such as Diabetes Mellitus (DM), which is associated with microvascular complications, causing increased MMP activity, directly or indirectly, due to oxidative stress. Objective: The aim of this study was conducted in order to assess wound healing using MMP-8 levels in GCF of diabetics with chronic periodontitis after diode laser assisted flap surgery. Methods: This interventional, comparative clinical trial, was conducted after obtaining approval from the Institutional Ethics Committee (IEC), (Study protocol number: 48/2020), and registered with Clinical Trials Registry of India (CTRI/2022/07/043898). Purposive sampling technique was used to select 30 patients with chronic periodontitis (15 systemically healthy patients, and 15 diabetic patients), who visited the Department of Periodontology. Results: Out of the 30 patients initially selected, 3 patients were lost to follow up and 1 patient was excluded from the study due to lack of compliance towards oral hygiene maintenance. Intragroup comparison of the clinical parameters at baseline and 3 months in both groups was statistically significant (p=0.000). This concludes that there was statistically significant improvement in the periodontal parameters of non-diabetic patients after diode laser assisted flap surgery. Inter-group comparison of the clinical parameters did not show statistical significance at baseline and at 3 months (p > 0.05). It can be concluded that there was comparable changes in the periodontal parameters in both groups after surgery. Conclusion: MMP-8 could be used as a futuristic tool for assessing wound healing especially in diabetics, so that necessary treatment interventions can be undertaken prior to development of any post-op complications. Laser assisted Modified Widman flap showed noteworthy improvement in the clinical parameters in both groups. Thus, proving that laser assisted MWF surgery is a favorable treatment modality, especially in diabetics who are immunocompromised and prone to infections. Keywords: GCF of Diabetics, Chronic periodontitis, Assessment of Wound Healing Using MMP-8 Levels in GCF.

1. BACKGROUND

Research has established periodontitis to affect overall systemic health, the World Health Organization estimated around 19% of the global adult population to be affected by periodontal disease, representing more than 1 billion cases worldwide (1), with diabetics being nearly three times more likely to develop the disease (2).

Inflammatory cytokines like Matrix Metalloproteinases (MMPs) are associated with the destruction observed in periodontal disease (3). There has been evidence of significant increases in MMP levels in patients with systemic disorders, such as Diabetes Mellitus (DM), which is associated with microvascular complications, causing increased MMP activity, directly or indirectly, due to oxidative stress (4).

MMP-8 (Collagenase 2 or neutrophil collagenase) are expressed mainly by polymorphonuclear leucocytes (PMN) (5). The triple helix structure of fibrillar collagen is split by MMP-8, and has affinity for type I and III collagen found in amply amounts in connective tissue of...
gingiva, and has been insinuated to be important in wound healing (6).

Currently clinical markers such as pocket probing depth are used to assess surgical outcomes. These markers represent healing outcome and not the ongoing healing activity. Wounds are subjected to various constructive and destructive factors, in which MMPs play a central role. As MMPs can degrade essentially every component of the ECM including proteoglycans, impaired regulation of these MMPs leads to faulty wound healing (7).

The inflammatory phase of wound healing (day 0-5) is characterised by infiltration of the wound by immune cells, like neutrophils, macrophages. Previous reports showed the suppression of innate immunity in the diabetics (8), and an increased amount of AGE deposition result in a decreased number of neutrophils migrating throughout from blood capillaries, thereby, delaying wound healing (9).

Understanding the molecular mechanisms of periodontal wound healing can provide a sound basis on which treatment strategies for prevention of delayed or impaired healing can be developed. Further translating this knowledge into today’s clinical treatment concepts can help clinician to formulate a specific treatment strategy (10).

2. OBJECTIVE

The aim of this study was conducted in order to assess wound healing using MMP-8 levels in GCF of diabetics with chronic periodontitis after diode laser assisted flap surgery.

3. MATERIAL AND METHODS

This interventional, comparative clinical trial, was conducted after obtaining approval from the Institutional Ethics Committee (IEC), (Study protocol number: 48/2020), and registered with Clinical Trials Registry of India (CTRI/2022/07/043898).

Purposive sampling technique was used to select 30 patients with chronic periodontitis (15 systemically healthy patients, and 15 diabetic patients), who visited the Department of Periodontology.

The inclusion criteria were, patients aged 30-50 years diagnosed with chronic periodontitis, with at least 3 adjacent teeth in the same quadrant, probing depth ≥ 5 mm, relative attachment level ≥ 6 mm, diabetic patients with HbA1C within 8%. The exclusion criteria were, smokers, pregnant and lactating patients, use of antibiotics in the 6 months prior to the study, patients with systemic conditions other than diabetes, patients who require regenerative or resective procedures. Patients who fulfilled these criteria were explained the study procedure in detail, and included in the study after obtaining their written informed consent.

Plaque index (Pl), gingival index (GI), parallel bleeding index (PBI), probing depth (PD), and relative attachment level (RAL) was measured for all the patients using UNC-15 probe (placement of the probe was standardized using a customized acrylic occlusal stent).

Phase 1 therapy was performed for all the patients, and oral hygiene instructions were given.

Patients were recalled after 2 months and their oral hygiene maintenance was assessed, those who had satisfactory oral hygiene and persistent pockets> 5mm were scheduled for surgery.

On the day of surgery, the selected sites were dried and isolated, and supragingival plaque accumulated on the gingival crevice was removed using a curette. A microcapillary tube (3µL) was placed at the entrance of the gingival crevice and a standard amount of GCF (3µL) was collected, contaminated samples were discarded. The GCF was then transferred into sterile centrifuge tubes containing 0.5mL of Phosphate Buffered Saline (PBS). The samples were stored at -20°C until assay. GCF samples were collected at 4 different time points, at baseline (day of surgery), 4 days, 7 days, 3 months post-surgery.

Surgical procedure (Figure 1 a-d)

The surgical area was anesthetized with 2% Lignocaine containing 1:80,000 adrenaline. A Modified Widman Flap (MWF) was raised, root surfaces were planed and degranulated using curettes. Undersurface of the flap was lasered using diode laser, having a wavelength of 808nm, and a power setting of 2 W, delivered using a flexible optic delivery system, in continuous, contact mode, using a ‘brush stroke’ motion on the inner surface of the flap to remove the pocket lining. The flap was thinned, papillae were trimmed, and tissue tags were removed. The flaps were then approximated using 3-0 non-resorbable black braided silk sutures.

Patients were given standard post-operative instructions, along with reinforcement of oral hygiene instructions. The patients were not prescribed any antibiotics. 500mg paracetamol tablets were given as rescue analgesic and patients were asked to make a note of the number of tablets consumed (if any). Patients were recalled on 4th day for GCF collection and 7th day for suture removal and GCF collection.

Patients were asked to maintain a diary evaluating post-operative pain or discomfort daily at night time for one week using a numerical rating scale (NRS)(11). This scale has markings from 0 to 10, where, 0 - No pain, 1 to 3 - slight pain, 4 to 6 - moderate pain, 7 to 10 - severe pain. The wound healing was checked using the Wound Healing Index (Landry et. al., 1988)(12) on the 4th and 7th day. Patients were kept on maintenance therapy with regular reinforcement of oral hygiene instructions. At the end of 3 months, all the clinical parameters were recorded.

Analysis of GCF samples:

The samples were assayed using Human MMP-8/ Neutrophil collagenase ELISA kit (KRISHGEN Systems), and the procedure was performed following the manufacturer’s instructions.

100 µl of prepared Standards and diluted Samples were added to the respective wells. The plate was then covered with a sealer and incubated at 37°C for 90 minutes, using Dry Shaker Incubator (Labnet, New Jersey, USA), the content was aspirated and washed with diluted Wash Buffer (1X).

100µL of diluted Biotinylated MMP-8 Antibody was added to each of the wells (Biotin Antibody Diluted at 1:100 in dilution buffer), plate covered with a sealer and incubated at 37°C for 60 minutes then washed. 100µL of diluted Streptavidin HRP Conjugate was added to each of the wells (Streptavidin HRP Conjugate diluted at 1:100 in Dilution Buffer) and mixed well, followed by incubation for 30 minutes at 37°C, and then washing the plate. 90µL of TMB substrate was pipetted into all the wells, and incubated at 37°C for 10
minutes. After incubation, it was observed that the solution turned bluish in colour. The reaction was stopped using 50 µL of stop solution to all the wells and a colour change from blue to yellow was observed. The colour developed was read at 450nm using multimode plate reader (PerkinElmer, Massachusetts, USA) within 15 mins.

**Statistical analysis**

The data obtained was tabulated in MS Excel and subjected to statistical analysis using SPSS software version 27. Independent samples t-test for intergroup and Paired t-test for intragroup comparisons of clinical parameters and MMP-8 levels were performed. Correlation between MMP-8 and WHI scores were analysed using Pearson’s correlation coefficient. P value < 0.05 was considered statistically significant.

### 4. RESULTS

Out of the 30 patients initially selected, 3 patients were lost to follow up and 1 patient was excluded from the study due to lack of compliance towards oral hygiene maintenance.

Intragroup comparison of the clinical parameters at baseline and 3 months in both groups was statistically significant (p=0.000). This concludes that there was statistically significant improvement in the periodontal parameters of non-diabetic patients after diode laser assisted flap surgery. Intragroup comparison of the clinical parameters did not show statistical significance at baseline and at 3 months (p > 0.05).

It can be concluded that there was comparable changes in the periodontal parameters in both groups after surgery (Table 1).

Intragroup comparison of MMP-8 showed statistical significance at all time points in both groups (Table 2). Intergroup comparison of MMP-8 levels in GCF showed statistical significance at baseline, 4th day, and 7th day post-operative (p < 0.05). MMP-8 levels at 3rd month post-surgery was not statistically significant (Table 3).

There was a marginal negative correlation between MMP-8 levels in GCF and Wound Healing Index (WHI) score on 4th day in both groups. However, this correlation is not statistically significant (Table 4). There was no significant correlation between NRS score on 4th day and WHI score on 4th day in both Diabetic group and non-Diabetic group.

### 5. DISCUSSION

A total of 30 patients were selected for the study, of whom, 15 were diabetic and 15 were non-diabetic, having moderate periodontitis and only horizontal defects (13), of which only 26 patients completed the study.

MMPs play an important role in wound healing, they are able to degrade all key components of the ECM, including those that promote cell adhesion, migration, and tissue remodelling in the wound (14). The increased MMP-8 levels seen in diabetic patients in our study was in accordance to that observed in other studies, this could be due to the rapid degradation of newly formed collagen in diabetics, leading to an impairment in wound healing (15).

In our study, MMP-8 was assessed on baseline, day 4, day 7 and 3 months. Intragroup comparison showed a significant increase in the MMP-8 level on the 4th day after surgery, and the 7th day after surgery, followed by a reduction observed at 3 months. This pattern was observed both in diabetic and non-diabetic groups. This is similar to a study where MMP-8...
levels in GCF and serum was assessed after recession coverage (16).

The increase observed in the 4th and 7th day could be due to neutrophils infiltrating the site of surgery to begin the task of wound debridement, to achieve this, the neutrophils release all the content in their granules, including MMP-8, this is usually seen around the 4th day after the wound is formed (16). Diabetes Mellitus (DM) is associated with microvascular complications, and diabetes mellitus (DM) causes increased MMP activity, whether directly or indirectly, due to oxidative stress, this could explain the significantly higher levels of MMP-8 observed in the diabetic group (17).

In the intergroup comparison, statistically significant difference (p value< 0.05) was noted on baseline, 4th day, and 7th day, with the diabetic group showing higher levels than the non-diabetic group (18). Kumar et al conducted a study where they evaluated MMP-8 and MMP-9 levels in gingival tissues of diabetic patients with chronic periodontitis, diabetic patients without periodontitis, and healthy patients. They reported that compared to patients without DM, patients with periodontitis and DM had twofold higher MMP-8 concentrations (18).

Wound healing was evaluated clinically using Wound Healing Index (12), which did not show any statistically significant difference, this could be due to the fact that the patients selected for this study had good glycaemic control (HbA1c<8%). Correlation of wound healing with MMP-8 levels, a negative correlation was found, which means, as the wound continued to heal, the MMP-8 levels reduced, however, this correlation was not of any statistical significance. This is similar to the study conducted by Jha et al, where MMP-8 levels were measured to assess wound healing after root coverage procedure (16).

Addition of Laser, especially the diode laser, to MWF towards treatment of periodontitis, presents with a wide range of potential benefits, such as, complete removal of diseased soft tissues, target the pathogenic micro-organisms, as well as stimulate wound healing (19). The diode laser used in this study was Sunny Diode (wavelength 808 nm, and power set-ting of 2.5W), delivered using a flexible optic delivery system used in continuous, contact mode.

Intragroup comparison of mean PI, GI, and BI scores in non-diabetic group and diabetic groups showed statistical significance (p=0.000). This is in accordance, with a study conducted by Chandra et al., where significant improvement was observed in the clinical parameters from baseline to 3 months, after using Laser as an adjunct to SRP (20).

Gokhale et al, had similar results, suggesting the effectiveness of surgical debridement of calculus and infected granulation tissue over non-surgical debridement, also the antisepsis mode of laser has the potential to break through the protective layer of the persisting biofilm, resulting in better antibi-otic effect compared to systemic antimicrobials (21).

Inter-group comparison of the baseline values of the periodontal parameters did not show statistical significance in PI (p= 0.85), GI (p= 0.76), BI (p= 0.77) at baseline, and at 3 months (PI had a p value of 0.174, GI had a p value of 0.206, BI had p value=0.381). Tervonen et al, Bay et al, and Sopi et al also found similar findings in their study, where they found no statistically significant difference in the bleeding index between diabetic and non-diabetic patients (22, 23). These results prove that diabetics and non-diabetics treated similarly for moderate periodontitis, show comparable improvement in plaque, gingival and bleeding scores, thereby, highlights that diabetics and non-diabetics have a similar response to periodontal surgery (24).

Occurrence of post-operative pain is a cause for concern in all patients undergoing surgical procedure. In our study, we asked all the patients to make a note of the post-operative pain using the NRS(11). No significant difference was observed between the two groups as the treatment modality was same for both groups. It is thought that by decreasing nerve sensitivity, laser therapy prevents pain signals from being transmitted from the injured site to the brain, resulting in a reduction in pain perception and an increase in endorphin and enkephalin production and release (natural pain-relieving chemicals) (25).

In a study to assess post-operative pain, Kolamala et al used theVAS scale. Patients’ pain perception and discomfort were recorded immediately after surgery, one day later, and one week later, and concluded that, the laser-treated sites experienced less pain than sites not treated with lasers(26). Study conducted by Sanz-Moliner et al to assess the effect of 810nm diode laser on post-operative pain, reported that post-operative complications were avoided after diode laser application, indicating that diode laser has no injurious effects when used in conjunction with MWF surgery (27).

Limitation of the study

The study is limited by a small sample size, short follow-up period. There was a lack of a control group treated with conventional flap surgery. Also, the effect of TIMP-1 on MMP-8 activity was not evaluated.

Future studies directed at using MMP-8 as a molecular marker for wound healing researched at various conditions, such as, different stages of periodontitis and types of periodontitis, even different treatment modalities using MMP-8.
to enhance wound healing especially in diabetics can be thought of. A recent study on rats using recombinant MMP-8 has shown encouraging results.(28). As literature is sparse regarding MMP-8 and wound healing in diabetics, this could lead to future studies and development of a chairside kit to monitor wound healing in diabetics as well as non-diabetics.

6. CONCLUSION

MMP-8 could be used as a futurist tool for assessing wound healing especially in diabetics, so that necessary treatment interventions can be undertaken prior to development of any post-op complications. Laser assisted Modified Widman flap showed noteworthy improvement in the clinical parameters in both groups. Thus, proving that laser assisted MWF surgery is a favorable treatment modality, especially in diabetics who are immunocompromised and prone to infections.

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