Estimation and Correlation of MMP-8 Levels in GCF and Serum with Wound Healing and Clinical Outcomes of Coronally Advanced Flap and Sub-Epithelial Connective Tissue Graft for Root Coverage - A Controlled Clinical Trial

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Abstract

Background and objectives: The success of any surgical procedure is predominately influenced by the pattern of its wound healing. The objective of the present study was to assess gingival crevicular fluid (GCF) and serum matrix metalloproteinase-8 (MMP-8) levels during the early healing phase of root coverage procedures. MMP-8 levels on days four and seven were correlated with the wound healing index (WHI) to evaluate the presence of MMP-8 during early post-surgical wound healing after root coverage procedures.

Materials and method: Fifteen isolated maxillary Miller's Class I/Class II recession defects in systemically and periodontally healthy patients in the age range of 25 - 57 years were treated with coronally advanced flap and sub-epithelial connective tissue graft (CAF + SCTG). GCF and serum samples were collected at baseline, day four, day seven and six months after surgery from the gingival sulcus of the recession defect. A contralateral tooth with clinically healthy gingiva was used as control and samples were collected from this site too at the same time intervals. MMP-8 levels in GCF and serum were measured using enzyme-linked immunosorbent assay (ELISA). Wound Healing Index was assessed on days four and seven. Mean root coverage (MRC) and complete root coverage (CRC) were assessed six months post-operatively.

Results: Statistically significant reduction in recession depth was observed with MRC of 88.67%. GCF and serum MMP-8 levels were significantly elevated on days four and seven post-surgery ($p < 0.001$) in the test site and reduced to baseline levels after six months. Weak positive correlation was observed between wound healing index and GCF MMP-8 levels on days four and seven. Moderate positive correlation was noted between serum MMP-8 levels and root coverage outcomes. However, this correlation was not statistically significant ($p > 0.05$).

Conclusion: The present prospective study showed satisfactory post-surgical healing and root coverage outcome. MMP-8 levels and its increase/decrease during the early wound healing follows the expected temporal pattern. No significant correlation was noted between MMP-8 levels during early wound healing and root coverage outcomes.

Keywords: MMP-8, wound healing, gingival crevicular fluid, gingival recession, coronally advanced flap, sub-epithelial connective tissue graft

Introduction

The success of any surgical procedure is predominately influenced by the pattern of surgical wound healing. Oral wounds heal faster compared to dermal wounds (Hakkinen *et al.*, 2000) and with reduced scar formation, but very little is known about the cellular and histological aspects of this process (Hammerle and Giannobile, 2014).

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The early phase of wound healing (days zero to five) involves blood clot consolidation, cellular activation and proliferation culminating in the regeneration of the periodontium. Impaired regulation of matrix metalloproteinases (MMPs) leads to inadquate wound healing (Nwomeh *et al.,* 1998, Nwomeh *et al.,* 1999). The function of collagenase during normal wound healing after periodontal surgery has been poorly studied (Chubinskaya *et al.,* 1996, Kuula *et al.,* 2009). MMPs constitute a family of zinc-dependent endopeptidases that mediate multiple functions both in the tissue destruction and immune responses related to periodontal inflammation, processing or degrading numerous extracellular, peri-cellular and non-matrix substrates. Under normal physiological conditions, the activities of MMPs are regulated by endogenous inhibitors (Visse and Nagase, 2003). Disruption of MMP regulation may result in diseases such as arthritis, cancer, atherosclerosis, aneurysms, nephritis, tissue ulcers and fibrosis (Pirila *et al.,* 2007). Collagenase-2 (MMP-8), also called neutrophil collagenase is expressed mainly by leukocytes of the polymorphonuclear (PMN) lineage (Weiss, 1989). It cleaves the triple helix of fibrillar collagen, has a strong affinity for type I collagen and type III collagen, but also degrades gelatin type VII, VIII, X collagen (Gurtner *et al.,* 2008) and is important during the acute phases of wound healing (Pirila *et al.,* 2007).

The coronally advanced flap combined with a subepithelial connective tissue graft (CAF+SCTG) is one of the most successful surgical procedures for recession management (de Sanctis *et al.,* 2011). Chronic wounds exhibit levels of MMPs 30 times greater than acute wounds. Inhibiting excessive protease expression in healing wounds may permit a favorable wound healing experience (Armstrong and Jude, 2002). Understanding the mechanisms of oral wound healing at a molecular level will pave the way to develop treatment strategies in preventing delayed or impaired healing (Sculean *et al.,* 2014). Analysis of MMP-8 in wound fluids is both a sensitive and specific assessment for inflammation. Therefore MMP-8 level analysis in GCF and serum will facilitate the early objective assessment of wound healing following root coverage procedures. This study aimed to assess the MMP-8 levels in GCF and serum during the post-operative healing stages following CAF+SCTG surgery for recession coverage.

Material and methods

Study design

This study was a human, prospective, single-center, single blind, clinical trial for the assessment of MMP-8 levels during the healing following reatment of Miller's Class I and II gingival recession defects. The MMP-8 levels were assessed in both GCF and serum at different intervals.

Patients seeking treatment for recession visiting the outpatient Department of Periodontology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore, India, and those satisfying the inclusion and exclusion criteria were selected for the study between November 2014 and July 2016.

Subject Selection

Fifteen isolated recession defects in systemically and periodontally healthy patients, satisfying the inclusion and exclusion criteria were selected for the study. Patients presenting with Miller's Class I, II or a combination in maxillary anterior, canines and premolar teeth with thick gingival biotypes (> 0.8 mm), and width of keratinized gingiva >1 mm were included in the study. Patients with aesthetic concerns, history of compliance to oral hygiene instructions and a full mouth plaque score of < 20% (O'Leary, 1972) were also included in the study. Exclusion criteria were medical history likely to influence the inflammatory response, e.g. atherosclerosis (Krupinski *et al.,* 2007), oral cysts (Wahlgren *et al*., 2001), inflammatory bowel disease (Pirila *et al.,* 2003), bronchiectasis (Prikk *et al.,* 2001), asthma (Prikk *et al.,* 2002) and diabetes, contra-indications for periodontal surgery, any type of regenerative periodontal therapy six months prior to the initial examination (da Silva *et al.,* 2004). Patients taking anti-inflammatory drugs were also excluded from the study. A detailed, thorough medical and dental history was obtained, and each patient was subjected to comprehensive clinical and radiological examination. The study protocol and informed consent form was approved by the institutional review board and ethical committee (KCDS 02-D012-54506) of Krishnadevaraya College of Dental Sciences and Hospital, Bangalore, India, affiliated to Rajiv Gandhi University of Health Sciences (RGUHS) and the trial was conducted in accordance with the principles of World Medical Association, Declaration of Helsinki (version 2008; clinicaltrials.gov. PRS number ID: NCT02863744). All patients were informed of the nature of the study, surgical procedure involved, potential benefits and risks associated with the surgical procedure and a written informed consent was obtained from all patients.

Interventions

Operator and Institution

All the surgical procedures were performed by a skilled clinician (JPG) in the Department of Periodontics, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore (India).

Clinical Measurements

Investigators

A single masked/blinded examiner (AJ) assessed all clinical measurements to ensure an unbiased evaluation. Prior to the study, the examiner was calibrated by the evaluation of gingival recession depth on two separate occasions on 10 patients. The examiner was calibrated to reduce error (>0.75) to establish consistency.

Data collection

The GCF samples were collected from gingival sulci of the teeth indicated for root coverage procedure (negative control) and from contra-lateral tooth with clinically healthy gingiva (positive control) at baseline and subsequent post-surgical follow-up (day four, day seven and six months). The serum samples were also collected at baseline and subsequent post-surgical follow-up (day four, day seven and six months).

Enrolled patients were scheduled for surgical treatment four weeks after their initial non-surgical therapy. The following clinical parameters were recorded to the nearest millimeter using a UNC-15-probe (University of North Carolina-15 periodontal probe; Hu-Friedy, Chicago, IL, USA) utilizing occlusal stents for positioning the probe at baseline and six months: gingival recession depth (GRD), gingival recession width (GRW), probing pocket depth (PD), clinical attachment level (CAL) and width of keratinized tissue (KTW).

Collection of Gingival Crevicular Fluid

The GCF samples were collected before any clinical parameter was recorded to avoid blood contamination. After gently drying the area with a blast of air, supra-gingival plaque was removed from the surface of the recession defect without touching the marginal gingiva with the help of a sterile curette. Samples of GCF were obtained by carefully inserting standard paper strips (Perio paper; Oraflow Inc., Smithtown, NY, USA) to a depth of approximately 1 mm into the sulcus for 30 seconds (Eren *et al.,* 2016; *Figure 1*). Strips contaminated by saliva or blood were discarded. The procedure was carried out carefully without any mechanical trauma. The GCF fluid volume on the standard paper strips were immediately measured using an electronic device (Periotron® 8000). The estimation of the actual volume (μl) was done according to the device instructions. The strips from the selected site were subsequently eluted into 200 μl of phosphate buffered saline, using a centrifugal machine (Remi House, Mumbai, India) vortexed and homogenized for 30 seconds and then micro-centrifuged at 3000 rpm at 4°C for 20 minutes and further stored in tubes (Eppendorf, Hamburg, Germany; Kurtis *et al.,* 2007). Eluates were then aliquoted into sterile tubes and stored at -80ºC until further analysis for the quantification of the MMP-8 using enzyme-linked immunosorbent assay (MyBiosource Inc, USA).

Serum sampling

Ten millimeters of blood were collected from each patient by venipuncture in the ante-cubital fossa using a 20-gauge needle with 2 ml syringe and were immediately transferred to the laboratory. Blood samples were collected into blood collection tubes containing no anticoagulant (Anil *et al*., 2013). The blood sample was allowed to clot at room temperature for one hour, serum was extracted from blood by centrifuging at 3000 rpm for 20 minutes. Serum samples were immediately transferred to tubes and stored at -80°C.

Pre-treatment procedures

Following enrolment, all patients received oral hygiene instructions to modify habits related to the etiology of gingival recessions which included coronally directed roll technique for brushing teeth. Initial cause-related therapy was completed four weeks prior to surgery (Pini Prato *et al.,* 2000).

Surgical procedure

Extra-oral antisepsis was performed using a 10% povidine iodine solution (Betadine, Purdue Pharma, Stamford, CT, USA) and intra-oral antisepsis was performed with pre-procedural mouthrinse of 0.2% chlorhexidine (Periex, Dentaids, India). Prior to the surgery, root surfaces of the recession defect were instrumented with curettes (Hu-Friedy, Chicago, IL) until smooth and hard root surfaces were obtained. Under local anesthesia (2.0% lignocaine, [Lignox 2%, Indoco Remedies Ltd, Goa, India] containing 1:80,000 adrenaline) an intra-sulcular incision was performed at the buccal aspect of the recession site. This incision was extended horizontally in a mesio-distal direction to dissect the buccal aspect of the adjacent papilla without involving the gingival margin of the adjacent teeth. From the mesial and distal extremities of this submarginal incision two oblique, divergent releasing incisions were extended beyond the mucogingival junction using a #15 blade (Bard Parker). The flap was raised starting from the submarginal incision and extended apically towards the alveolar bone crest. The elevation was extended in mesio-distal and apical direction to facilitate passive coronal advancement of the surgical flap. The adjacent papillae were de-epithelialized using a #15 blade (*figure 1C*). The freely movable flap was positioned coronally to extend beyond the cemento-enamel junction (CEJ) (Pini Prato *et al.,* 2000). The flap mobilization was considered adequate when the marginal portion of the advanced flap was passively reaching a level 1 to 2 mm coronal to the CEJ, in the surgical area (Pini Prato *et al.,* 1999). After preparation of the recipient bed, the connective tissue graft was harvested from the palate, using single incision technique (Hürzeler and Weng, 1999). The connective tissue graft was placed on the root surface at the level of the CEJ and then secured with 4-0 absorbable sutures (Vicryl, Ethicon, Somerville, NJ, USA) using sling suturing technique (Pini Prato *et al.,* 2000) (*figure 1D*). To permit the coronal advancement of the flap margins at least 1 to 2 mm coronal to CEJ of indicated tooth, all muscle insertion present in the thickness of the flap was eliminated. Interrupted sutures were placed on releasing incisions in an apico-coronal direction using 4-0 silk (Mersilk, Ethicon, Somerville, NJ, USA) sutures (*figure 1E*).

Post-operative medication

Ibuprofen 400 mg (Brufen, Knoll Pharmaceuticals (S.A.) Pty Ltd) was prescribed three times daily for three days as post-operative analgesic (Zucchelli *et al.,* 2010). Chlorhexidine mouthwash (0.2%; Peridex, Dentaids, India) was prescribed twice daily for 14 days. Patients were asked to avoid any trauma for two weeks in the surgical area and were recalled after 14 days of surgery for suture removal. Recall and reinforcement of oral hygiene instructions was done every two months.

GCF samples from the surgical site and serum samples were collected on days four (*Figure 1F)* and seven post-surgery (*Figure 1G*). The dressing was removed from the surgical site and a GCF sample was collected as described above. The area was irrigated with saline and dressing was replaced.

MMP-8 analysis was carried out using sandwich ELISA technology according to manufacturer's guidelines. The change in color was measured spectrophotometrically at a wavelength of 450 nm. The concentration of MMP-8 in the samples was then determined by comparing the optical density of the samples to the standard curve. Results were expressed as ng/ml for each sample.

Post-surgical protocol

After two weeks post-surgery, sutures, pack and surgical stent were removed. Patients plaque control regimen were reinforced using chlorhexidine rinsing two times a day, mechanical tooth cleaning of the treated tooth using an ultra-soft tooth brush and a roll technique. Patients were recalled on days four and seven and six months for follow up (*Figure 1H, 1I).*

Wound healing analysis

Wound healing index (WHI; Huang *et al.,* 2005) was recorded on days four and seven.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for Software and Social Science, version 22). Each surgical site was considered independently to statistically assess the MMP-8 expression in healing outcome. The study was powered to detect minimum clinically significant difference in root coverage of 1 mm using $p \leq 0.05$ and 95% power (G power software analysis).

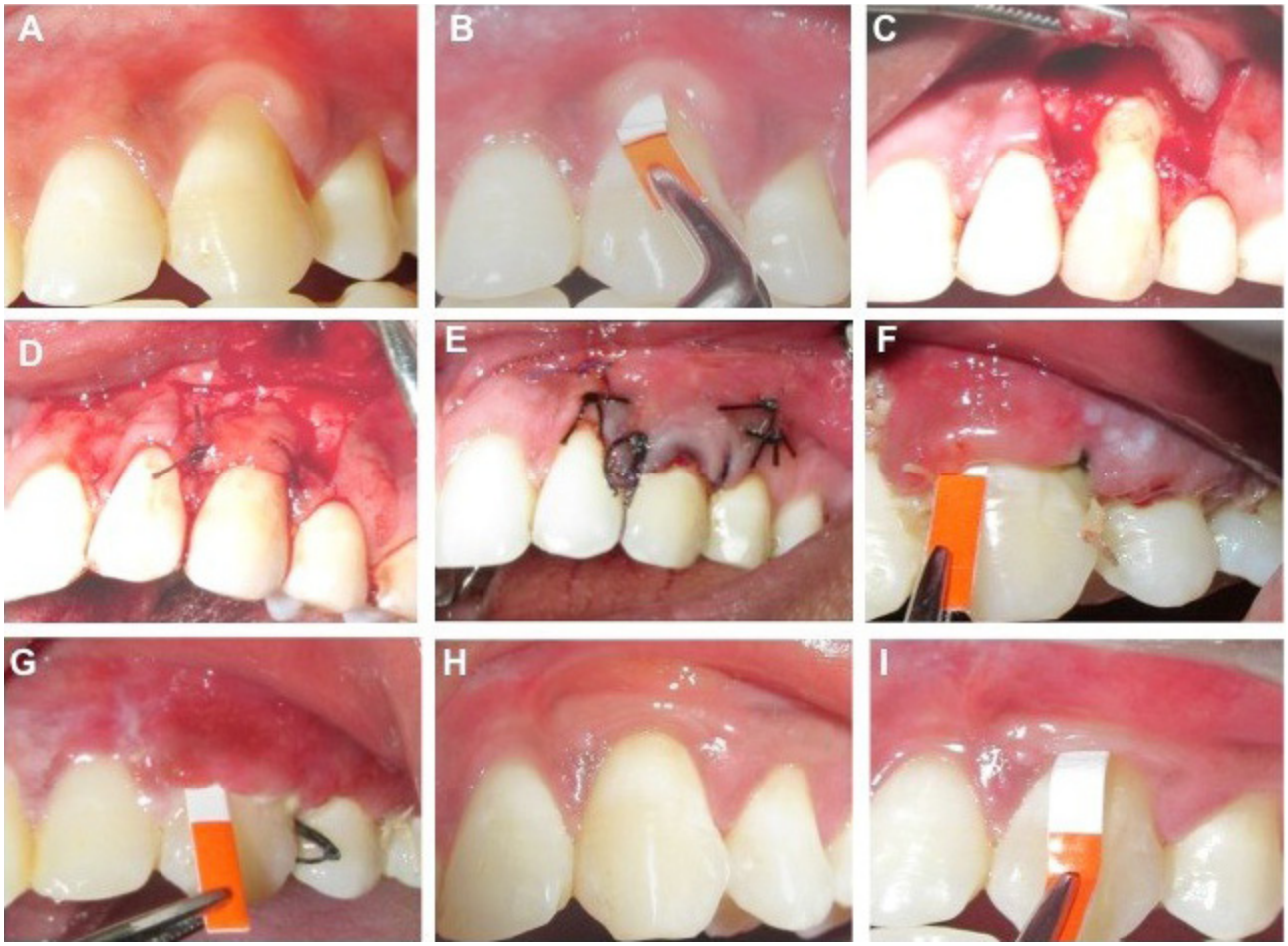

Figure 1 - Surgical Procedure and GCF sample collection at test site

A. Pre operative.
B. GCF sample collection (baseline)
C. Preparation of recipient site
D. Stabilisation of connective tissue graft
E. Suturing
F. GCF sample collection (Day 4)
G. GCF sample collection (Day 7)
H. 6 months post operative
I. GCF sample collection (6 months)

Repeated measure one-way analyses of variance were used to test the GCF and serum MMP-8 levels between various time points. A paired- "t" test was performed to compare the GCF and serum MMP-8 levels between the test and control group at different time intervals. Wilcoxon signed-rank test was used to compare clinical parameters before and after the treatment. The Pearson correlation coefficient was calculated to measure the strength of the linear relationship between two variables (GRD and MMP-8 levels). Receiver operating characteristic (ROC) curve were generated by plotting sensitivity versus specificity and area under curve values were assessed to predict the value of MMP-8 as biomarker of wound healing.

Results

All of the 15 patients enrolled completed the clinical trial and scheduled follow up. In the test group the GCF MMP-8 level increased three-fold from 13.21 ± 1.30 ng/ml at baseline to 40.02 ± 4.46 ng/ml and decreased to 29.94 ± 4.11 ng/ml on day seven. The change in MMP-8 levels at different time intervals was statistically significant. GCF MMP-8 level returned to baseline values at six months. In the healthy site no significant change in GCF MMP-8 levels were noted at all time intervals studied (*Figure 2*). GCF volume was comparable between the test and control at baseline, statistically significant difference in GCF volume levels was noted at various time intervals for test site (*Figure 2*), whereas no significant change was noted for the control site.

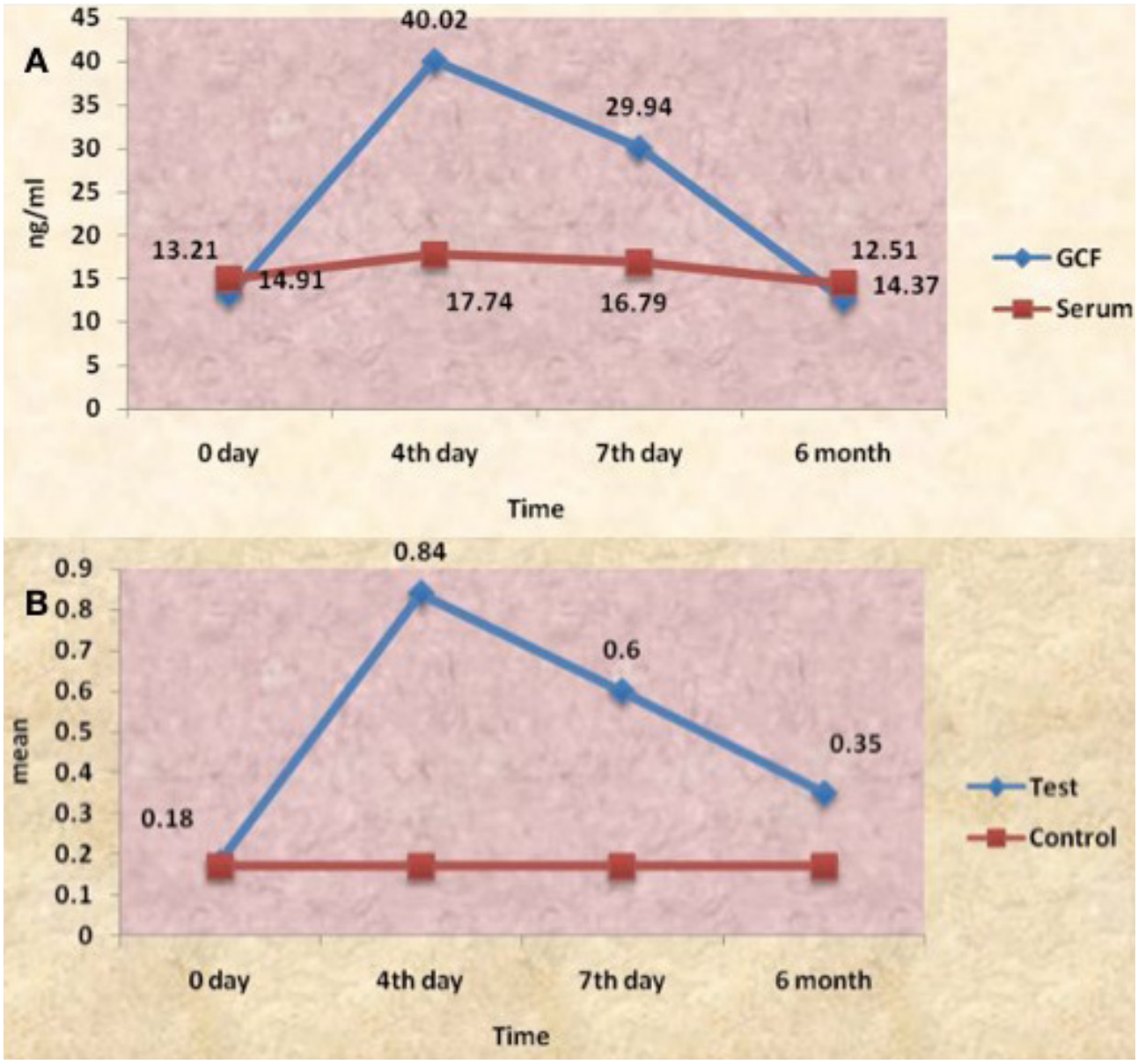

Figure 2: MMP-8 levels in GCF and serum and GCF volume at different time intervals
A. MMP-8 levels in GCF and serum at different time intervals
B. GCF volume at different time intervals
Repeated measures ANOVA, Bonferroni post hoc test, *p <0.05 statistically significant, -> 0.05, non-significant, NS

At the day four post-surgical follow up, a weak positive correlation was observed between the GCF MMP-8 levels and wound healing index. By day seven there was only a very weak negative correlation between GCF MMP-8 level and wound healing index with 'r' value of -0.05 (*p* value of 0.85) that was statistically not significant. On day four post-surgical follow up, a weak negative correlation was observed between serum MMP-8 level and wound healing index. Similarly on day seven a weak negative correlation was observed between serum MMP-8 level and wound healing index. Individual GCF and serum MMP-8 levels on days four and seven were not statistically significant as being predictive of wound healing outcome.

WHI significantly reduced from day four to day seven in the test site (*Table 1*). There was a statistically significant reduction in GRD and GRW at six months (*Table 1*). 88.67% of mean root coverage (MRC) and 67% of complete root coverage (CRC) was observed. KTW and CAL gain was statistically significant (*Table 1*).

MMP-8 levels in GCF and serum at baseline showed statistically insignificant correlation (*Figure 3*). Moderate positive correlation between GCF/serum MMP-8 level and gingival recession depth reduction was noted (*Figure 3*). No significant correlation between GCF/serum MMP-8 levels and wound healing index was observed.

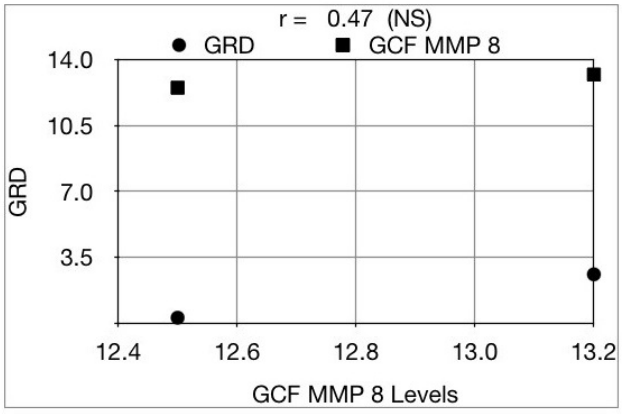

Figure 3: Correlation between MMP-8 levels in GCF and recession reduction
GRD: gingival recession depth (mm), Spearman correlation test, , *p <0.05 statistically significant, -> 0.05, non-significant, NS

Weak positive correlation of no statistical significance was noted between MMP-8 levels in GCF and serum at baseline and day four post-surgical follow up. A statistically significant moderate positive correlation was noted on day seven post-op review, whereas a very weak positive correlation was noted at the six month follow up.

Discussion

Post-surgical healing pattern is highly regulated by matrix degrading enzymes (Nwomeh *et al.,* 1998; Nwomeh *et al.,* 1999). In the current study, CTG treated sited sites showed higher levels of MMP-8 that sequentially increased from baseline to day four (three-fold) and gradually decreased by day seven (2.1-fold) and returned to baseline value by six months. After surgery, neutrophils infiltrate the wound site during the acute inflammatory phase of healing and begin the task of wound debridement and decontamination, which includes the actions of collagenases such as MMP-8. The increased GCF MMP-8 level observed from baseline to days four and seven was most likely due to increased numbers of PMNs and collagenolytic activity in the tissue treated with CTG.

Nwomeh *et al.* (1998) studied tissue extracts periodically from occlusive bandages of acute open human dermal wounds and noted that the levels of MMP-8 peaked at day four and persisted for around a week. The increase in the fourth day may also be related to increased fibroblast activation (Wikesjo *et al.,* 1991). In keeping with this temporal pattern of the MMP-8 expression, the current study assessed the MMP-8 profile on days four and seven. MMP-8 level increased during days four and seven as compared to baseline values. This is in accordance with similar studies of dermal wounds. In the later phase of healing, a predominance of type-III collagen occurs and as the rate of remodeling reduces, the level of MMP-8 also reduces. This stage of wound healing is regulated by a number of MMPs and tissue inhibitor of metalloproteinase. Nwomeh *et al.,* (1998) observed a 10 to 100-fold increase in MMP-8 levels in chronic dermal non-healing wounds. In the present study, MMP-8 levels in the GCF increased three-fold. No patients presented with delayed or healing complications. Said *et al.,* (1999) also noted a three-fold increase in MMP-8 levels from baseline value to day four when GTR therapy for periodontal regeneration was done.

Table 1. Changes in Clinical Parameters after six Months

Parameters	Test (CAF + SCTG) BL-6M	*p* - value
Recession depth reduction (mm)	2.37 ± 0.79	0.001*
Recession width reduction (mm)	2.87 ± 0.63	0.001*
Gain in keratinized tissue width (mm)	1.84 ± 0.78	0.001*
CAL gain (mm)	2.17 ± 0.79	0.001*
Complete root coverage (%)	67%	
Mean root coverage (%)	88.67%	
Wound healing index	1.73 ± 0.46	0.008*

Wilcoxon sign rank test,*p < 0.05 statistically significant

Similar results were observed by Eren *et al.,* (2016) where a 2.1-fold increase in MMP-8 was observed on the tenth day in root coverage by CAF+SCTG. The current study reflects similar temporal patterns of a 2-3-fold increase in the MMP-8 levels in wound fluid by day four that then decreases by day seven until at six months post-surgery levels return to those observed at baseline. The orchestrated increase and decrease of MMP-8 levels suggests that MMP-8 is actively involved in the early wound healing phase following CAF+SCTG procedures for root coverage.

Gutierrez-Fernandez *et al.* (2007) observed a significant delay in wound closure in *MMP8*−/− mice and an altered inflammatory response in their wounds, with a delay of neutrophil infiltration during the first days and a persistent inflammation at later time points. The present study reiterates that the presence of normal MMP-8 levels in patients facilitates wound healing without complications such as delayed/non-healing wounds.

A weak positive correlation was observed between GCF MMP-8 level and WHI on day four, whereas on day seven a weak negative correlation was observed. GCF MMP-8 levels were closely related to wound healing. No similar studies have been carried out and hence the nature of this correlation cannot be compared. The pattern of GCF MMP-8 levels follows the inflammatory profile of WHI as it increases on day four during the peak of inflammation and gradually decreases as the wound heals and matures. By six months, the mean GCF MMP-8 level was reduced to baseline value which confirms the presence of stable, mature connective tissue matrix at the treated site. This observation is comparable to that of Eren *et al.,* (2016) where levels of MMP-8 after six months reduced to baseline values in CAF+SCTG group. Similar results were reported by Said *et al.,* (1999) where the levels of MMP-8 after three months reduced to baseline level after surgical treatment of intra-bony defect. In the present study at baseline, the mean serum MMP-8 level obtained was similar to the study by Lauhio *et al.,* (2016) in healthy individuals. When serum MMP-8 level was correlated with WHI on days four and seven, a weak negative correlation was observed, suggesting that the current trial did not show evidence of an association between surgical gingival wound healing and serum MMP-8 levels.

MMP-8 levels in GCF and serum at baseline, day four, day seven and six month follow up period showed weak positive correlations. However, they were not statistically significant. No similar studies have been carried out and hence the nature of this correlation cannot be compared. The local influence of wound healing does not seem to affect the serum MMP-8 levels as much as GCF MMP-8 level.

Six months post-surgery, treatment resulted in mean root coverage of 88.67% similar to the results found by Cortellini *et al.* (2009) 83.3% MRC, Jankovic *et al.* (2010) 91.9%, Keceli *et al.* (2015) 79.9%, Lops *et al.* (2015) 83.3%. Complete root coverage of 67% was achieved as also reported by Mahajan *et al.* (2012). A moderate positive correlation was noted between gingival recession depth and GCF MMP-8 level, however this was not statistically significant.

There was a weak negative correlation between serum MMP-8 levels and recession depth and width reduction. Serum MMP-8 levels in the current study showed no indicative role in recession coverage outcome. Since there is no information available in the literature to compare the co-relation between MMP-8 levels and root coverage outcomes, no conclusive remarks can be made.

In the present study, the six month follow up period was chosen for collection of GCF and serum post surgically as this period is considered adequate to provide soft tissue maturity and stability as reported in systematic reviews (Rosetti *et al.,* 2000, Cairo *et al.,* 2008). However long-term studies are required to assess root coverage outcomes, as grafted sites over long-term period show tendency for coronal shift of the gingival margin (Cortellini and Pini Prato, 2012). For graft harvesting, the single incision technique by Hurzeler and Weng (1999) was selected, as blood supply for the overlying flap remains un-compromised facilitating primary intention healing of the donor site. In the present study it was observed that patients with higher level of MMP-8 at baseline reported with partial root coverage. However, this association was not statistically significant. The results of the present study showed that the difference in the levels of MMP-8 at different time points during the early stages of healing had a weak positive association with the wound healing index.

The current study is one of the few studies that have assessed MMP-8 levels in GCF and serum post CAF+SCTG for recession treatment. The study has also corelated these values with wound healing outcomes as assessed by WHI and root coverage. In the future patients with increased MMP-8 levels at baseline could be strategically managed following root coverage treatment procedures to optimize their treatment outcome. Such a treatment option would be based on the concept that can increased MMP-8 levels may impair surgical outcomes for the management of gingival recession. Thus during surgical treatment, addition of biologic modifiers agents that might promote early wound healing by elevating TIMP-1 levels and suppressing MMP-8 may be of benefit (Eren *et al.,* 2016).

One of the major limitations of this study is relatively small study population. Six month follow up is also quite short and could be considered another limitation. The present study enrolled only a single group of systemically healthy patients presenting with normal baseline levels of MMP-8. A comparison with another group that has higher MMP-8 levels at baseline and their treatment outcome should also be assessed.

Conclusion

The present study showed MMP-8 levels were higher on days four and seven than at baseline and reduced to baseline values at 6 month post-surgical follow up. Recession reduction and GCF MMP-8 level showed a weak positive correlation. A positive correlation between GCF MMP-8 levels and WHI was observed and this suggests a role for MMP-8 in the early stages of wound healing.

There is a need for long term randomized controlled clinical trials comparing GCF and serum MMP-8 levels with commonly used surgical procedures for root coverage of recession defects. Furthermore the use of biological modifiers or immune-modulators that can reduce MMP levels facilitate early and rapid wound healing and thus decrease patient postoperative pain and discomfort.

Conflict of interest and source of funding

The authors received no financial support for the conduct of the study. The authors declare that there is no conflict of interest concerning the contents of the study. This study has been self-supported by the authors.

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