Original Article

A quantitative analysis of macrophage–colony-stimulating factor in peri-miniscrew implant crevicular fluid before and after orthodontic loading

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ABSTRACT

Objectives: To analyze macrophage–colony-stimulating factor (M-CSF), a bone remodeling biomarker in the peri-miniscrew implant crevicular fluid (PMICF) after insertion and orthodontic loading.

Materials and Methods: This prospective study included 40 miniscrew implant (MSI) sites in 10 subjects undergoing fixed orthodontic mechanotherapy utilizing miniscrew anchorage. After dental alignment, miniscrews were inserted between the second premolar and first molar roots. After 21 days of insertion, MSIs were direct loaded with closed-coil springs (200 g force) for en masse retraction of anterior teeth. PMICF was collected with Periopaper™ strips from the gingival crevice around MSIs at six time points (T1–T6: 1 hour, 1 day, 21 days postinsertion, and 7, 21, and 42 days postloading). PMICF was quantified for M-CSF by enzyme-linked immunosorbent assay. Paired comparison of mean M-CSF concentrations before and after loading stages (T1–T6) was made using the Wilcoxon signed-rank test.

Results: The mean M-CSF concentration showed a significant peak at T3 (21 days postinsertion; 12.646 pg/mL; T1 vs T3: $P < .0001$). After orthodontic loading of miniscrews, M-CSF levels increased to 13.570 pg/mL at T4 (7 days after loading; T1 vs T4: $P < .001$) and maintained at a plateau to T5 (21 days postloading; 11.994 pg/mL). However, the difference between preloading and postloading was not statistically significant (T3 vs T4).

Conclusions: The maximum M-CSF activity around MSIs was observed at around 3 weeks of miniscrew insertion, suggesting underlying bone remodeling after surgical injury. However, orthodontic force on MSIs did not cause any significant surge in M-CSF levels postloading. (*Angle Orthod.* 0000;00:000–000.)

KEY WORDS: Macrophage–colony-stimulating factor; Miniscrew implant; Bone remodeling; Peri-miniscrew implant crevicular fluid; Biomarker

INTRODUCTION

As an anchorage device, the miniscrew implant (MSI) has attained prominence in current orthodontic practice. However, MSI stability has always been a concern for the orthodontist.

The failure rate of MSIs was reported at 14.3% (95% confidence interval [CI], 11.5–15.9%) in a recent metanalysis.[1] There are multiple factors, such as insertion site, keratinization of attached gingiva, bone quality, loading protocol, and insertion technique, that influence the stability of temporary anchorage devices.[2] Recently, research[3] on the biological response around the implant site has been an area of significant investigation.

During MSI insertion, the peri-implant soft tissue and alveolar bone undergo surgical injury, with microcrack propagation in the bone around the MSI, which usually follows a healing process.[4,5] While alkalization around the surgical injury site promotes bone healing, contin-

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ued inflammation leads to implant mobility and rejection.[6] Implantation provokes an inflammatory response, leading to the release of growth factors and cytokines by interstitial cells. The release of interleukins and tumor necrosis factor–alpha (TNF-α) by matrix cells promotes activation, recruitment, and proliferation of macrophages and other cell populations.[7] The cytokines (interleukins, TNF-α) modulate osteoclastogenesis directly by increasing RANKL and macrophage–colony-stimulating factor (M-CSF) production.[7] Osteoclast precursor cells express RANK and c-Fms (M-CSF receptor). Upon stimulation by these two essential factors RANKL and M-CSF, differentiate to mature osteoclasts, resulting in bone remodeling around the MSI.[8] M-CSF causes the recruitment of myeloid cells to the site of injury. On binding to its c-Fms receptor on osteoclast precursor cells, M-CSF triggers distinct signaling pathways that promote the proliferation, differentiation, and persistence of osteoclasts, thus facilitating osteoclastogenesis.[9]

Peri-miniscrew implant crevicular fluid (PMICF) is a serum exudate analogous to gingival crevicular fluid (GCF). PMICF carries biomolecules reflecting underlying cytocellular activity of the tissue around the MSI.[10] Inflammatory biomarkers with specific roles, such as interleukins (IL-1β, IL-2, IL-6, IL-8), growth factors, TNF-α, RANKL, chondroitin sulfate, osteoprotegerin, TGF-β, and osteocalcin in PMICF, have been studied previously[11–16] to assess the host response to MSI insertion and orthodontic loading forces.

M-CSF, a bone remodeling biomarker, is known to have pro-inflammatory osteoclastic activity. RANKL and M-CSF mediate osteoclastic bone remodeling around prosthetic implants.[17] There is a lack of literature evaluating M-CSF as a biomarker for assessing bone remodeling around MSIs in humans.

This study evaluated M-CSF in PMICF at various time points to provide a trendline of its concentration around MSIs before and after loading with orthodontic force. A qualitative analysis of M-CSF in PMICF will likely enhance understanding of bone remodeling around MSIs.

MATERIALS AND METHODS

Study Population

This prospective study included 40 miniscrew implant sites in 10 patients (eight females, two males; age: 16–24 years) planned for fixed orthodontic mechanotherapy with all first premolar extractions utilizing direct anchorage with MSI. A convenience sample of 40 MSI sites was studied as preliminary research to understand the pattern of biomarker alterations at different time points. All chosen subjects had a full complement of teeth up to the second molars, with healthy periodontium and no significant medical history, drug history, or metabolic bone disease. Informed consent was obtained for sample collection and treatment from all subjects participating in the study. The rights of the human subjects were protected, and approval was obtained from the Institute Ethics Committee for Postgraduate Research, All India Institute of Medical Sciences, New Delhi (IECPG-65/27.11.2015).

Intervention

Levelling and alignment of the dental arches were attained to a stage of passive placement of 0.019 $\times$ 0.025-inch stainless-steel archwire in a 0.022 $\times$ 0.028-inch bracket slot appliance. A self-drilling miniscrew implant (8 $\times$ 1.5 mm; Tomas®, Dentaurum, Germany) was inserted interdentally between the second premolar and first molar at the level of attached gingiva at anchorage locations.[14,18] MSIs were directly loaded with closed-coil springs (nickel-titanium [NiTi], 9-mm length, 200 g force) for en masse retraction of anterior teeth (Figure 1a) at 21 days after placement.[19] The PMICF sample was collected from crevice around miniscrews at six time points with Periopaper™ (1.2 μL; Oraflow Inc, Smithtown, NY) (Figure 1b). The six time points were as follows: T1 (1 hour postinsertion), T2 (1 day postinsertion), T3 (21 days postinsertion), T4 (7 days postloading), T5 (21 days postloading), and T6 (42 days postloading). After sample collection, Periopaper strips were stored in 100 μL of phosphate-buffered saline (PBS) in an Eppendorf tube at -80°C. Biological marker M-CSF was quantified by biochemical analysis using an enzyme-linked immunosorbent assay (ELISA).

Biochemical analysis of M-CSF employed the Human M-CSF ELISA Kit (RayBio® RayBiotech Life Inc, Peachtree Corners, Ga). Stored samples were brought to room temperature and vortexed. After dilution with distilled water, the sample strip was pipetted into wells, to which antibody to biomarker was pipetted. After incubating mixed samples and reagents for fixed intervals, a biotinylated anti-human M-CSF antibody was added. After the unbound antibody was washed, horseradish peroxidase–conjugated streptavidin was added to the wells. The color developed proportionately to the concentration of bound M-CSF, the optical density of which was measured at 450 nm.

Statistical Analysis

SPSS software (version 20.0; IBM, Armonk, NY) was used to analyze data. Data were checked for normality using the Shapiro-Wilk test. Mean M-CSF concentration in PMICF was calculated and plotted against time

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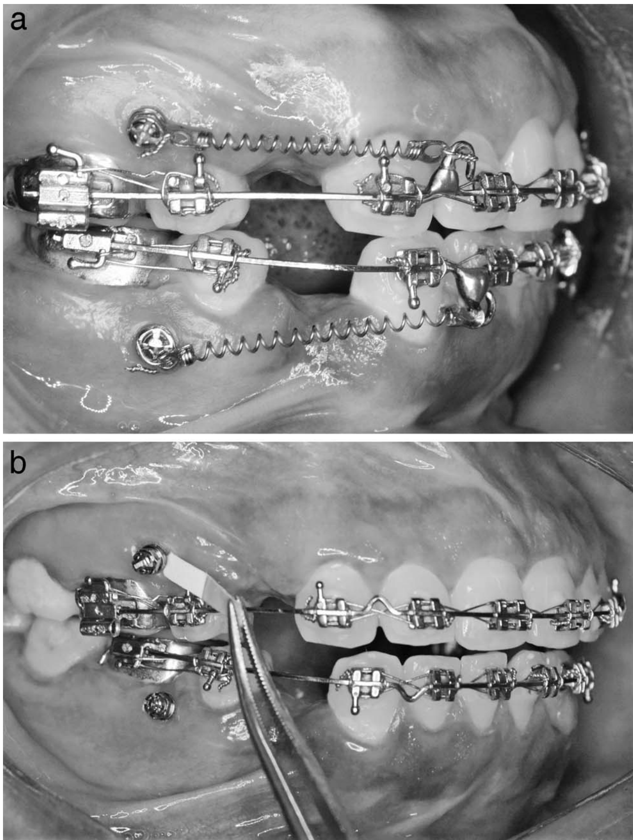

Figure 1. (a) Interradicular miniscrews loaded with NiTi closed-coil springs attached to hooks soldered on archwires for en masse retraction of anterior teeth. (b) Peri-miniscrew implant crevicular fluid (PMICF) sample collection with Periopaper™.

points (T1–T6) to obtain a trendline for the entire period of study. Paired comparisons of M-CSF concentration at the time points (T1–T6) were achieved using a nonparametric Wilcoxon signed-rank test. Mean M-CSF concentrations ($\pm$ standard deviation) from all subjects were plotted again at six time intervals preloading and postloading.

RESULTS

Of the 40 MSIs, 32 MSIs were stable throughout the study period. Only successful MSIs could be included in the analysis for 63 days. The mean M-CSF concentration in PMICF 1 hour after MSI insertion (T1) was 8.891 pg/mL, which remained low until 24 hours postinsertion (T2). The levels increased to a peak value of 12.646 pg/mL at T3 (21 days post-insertion; $P < .0001$). After orthodontic loading of miniscrews, the M-CSF concentration in PMICF increased to 13.570 pg/mL at T4 (7 days postloading) (T4, $P < .001$, T1 vs T4), remained elevated until T5 (21 days postloading), and declined after that.

The M-CSF concentration in PMICF showed peak activity at 21 days postinsertion (T3) and 7 days post–orthodontic loading (T4), followed by a plateau phase until T5, and then a decline to baseline. However, the difference in M-CSF levels preloading and postloading was not significant (T3 vs T4, $P > .05$). The mean M-CSF concentration in PMICF at all time points was compared for the maxilla and mandible, and its trendline was similar for both jaws (Figure 2; Table 1).

Table 1. Descriptive Statistics and Comparison of Macrophage–Colony-Stimulating Factor (M-CSF) Concentration in Peri-Miniscrew Implant Crevicular Fluid (PMICF) Between Time Points (T1–T6) Before and After Loading[a]

Time Points	Mean M-CSF Concentration in PMICF, pg/mL ($\pm$SD)	P50 M-CSF Concentration in PMICF, pg/mL (IQR)	Paired Comparison of Mean M-CSF Concentration Before and After Loading (T1–T6)[b]
T1	8.891 $\pm$ 3.3	9.236 (5.928)	
T2	9.569 $\pm$ 4.1	9.473 (5.204)	T2 vs T1 (ns)
T3	12.646 $\pm$ 5.1	12.842 (7.447)	T3 vs T1****
T4	13.570 $\pm$ 6.7	11.477 (7.677)	T4 vs T1***
			T4 vs T3 (ns)
T5	11.994 $\pm$ 5.1	10.378 (7.239)	T5 vs T1**
T6	8.949 $\pm$ 2.9	8.892 (3.371)	T6 vs T1 (ns)

[a] T1 indicates 1 h postinsertion); T2, 1 d postinsertion; T3, 21 d postinsertion; T4, 7 d postloading; T5, 21 d postloading; T6, 42 d postloading; SD, standard deviation; IQR, Interquartile range; and MSI, miniscrew implant.
[b] Wilcoxon signed-rank test.
* $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$; **** $P \leq .0001$; ns $\geq .05$.

DISCUSSION

A significantly elevated bone resorption biomarker (M-CSF) level was observed on the 21st day post-insertion and the seventh postloading, suggestive of osteoclastic activity around MSIs at these time points associated with peri-implant bone turnover. A similar finding was reported by Ure et al.,[20] in whose study implant stability quotient values decreased in the first 3 weeks following implant insertion and increased after that. The decrease in stability can be associated with active resorption or remodeling of bone around the implant.

Studies in the literature on biomarkers of inflammation and remodeling in PMICF have reported peak concentrations of TNF-α,[13] IL- 2, IL-8,[12] and cell-free nucleic acids[21] at 24 hours post–MSI loading. Monga et al.[11] demonstrated an increase in IL-1β at 4 hours postinsertion and 24 hours after loading. Most of these pro-inflammatory markers rise early in PMICF within 24 hours of insertion. A few studies estimated bone remodeling biomarkers around miniscrews. Enhos et al.[14] reported a substantial increase of RANKL in PMICF for immediate loading MSIs compared to unloaded miniscrews at all periods. TGF-β, known to stimulate M-CSF–induced osteoclastogenesis, was

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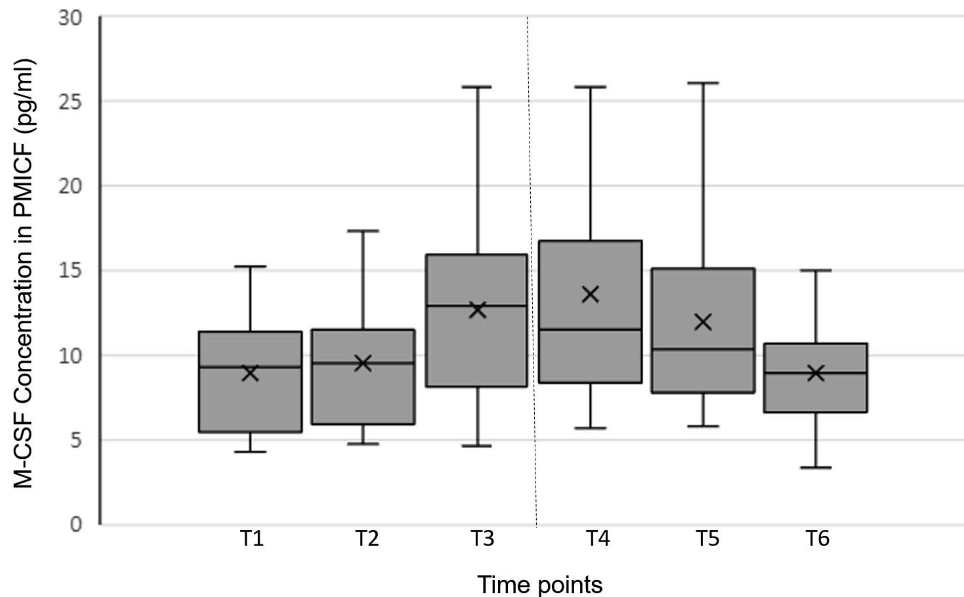

Figure 2. Graph depicting mean M-CSF concentration in PMICF from T1 through T6. Mean M-CSF level significantly increased at T3, T4, and T5 and declined to baseline by T6. M-CSF, Macrophage–colony-stimulating factor; PMICF, peri-miniscrew implant crevicular fluid; T1, 1 hour postinsertion; T2, 1 day postinsertion); T3, 21 days postinsertion; T4, 7 days postloading; T5, 21 days postloading; and T6, 42 days postloading. Dotted line depicts loading of MSI with NiTi closed-coil spring after 21 days of insertion. (x) in bar depicts mean M-CSF concentration. Solid line in bar depicts P50 M-CSF concentration.

elevated in PMICF soon after miniscrew insertion (1 hour).[22]

Miniscrew insertion leads to microhemorrhage and microcracks in the bone around it, followed by remodeling that involves both osteoclastic and osteoblastic activity in a coupled manner.[5] Microcrack propagation occurs primarily as a result of the difference in the elasticity of bone and the MSI, leading to initiation of the biologic response.[4] The microcracks are repaired by microcallus formation and subsequent mineralization initiated on the region's nuclei of calcium phosphate crystals.[23] The microhemorrhage around the MSI triggers activation and degranulation of platelets, thereby inducing several inflammatory cascades, which cause the release of pro-inflammatory mediators (eg, TNF-α), known to promote osteolysis by stimulating M-CSF gene expression.[17] For this reason, the initial rise in M-CSF can possibly be attributed to inflammation following the surgical trauma of implant placement and induction of osteoclastogenesis by pro-inflammatory cytokines.

An increase in the concentration of M-CSF in PMICF at T4 (ie, 7 days after loading) in the current study is suggestive of bone remodeling activity around MSIs after applying orthodontic force by NiTi coil springs for en masse retraction. However, the difference between the preloading and postloading concentrations of M-CSF in PMICF was not significant. These observations supported that orthodontic loading with 200 g springs will not compromise MSI stability, and that is experienced clinically in successful cases.

Bone remodeling cycles have three phases: bone resorption, reversal, and bone formation. As the bone reversal phase follows the bone resorptive phase around MSIs, a significant decline was seen after 21 days of MSI loading, reaching baseline, similar to levels at T1. This response may be attributed to the resorption of the callus around the MSI and replacement by new trabecular bone during bone remodeling around the implant.

The findings of this study were in agreement with the results of previous research. Zhang et al.[24] found upregulation of matrix metalloproteinases (MMPs) in GCF at 1 to 4 weeks after orthodontic force application, indicating extracellular matrix degradation associated with bone remodeling. Sarahrudi et al.[25] reported a 2.5-fold increase in M-CSF levels in fracture hematoma of the rabbit femur and systemic venous blood at weeks 1 and 2 after the fracture. This emphasizes M-CSF's active role in bone healing. Kaku et al.[26] reported M-CSF mRNA expression in mice osteoblasts and fibroblasts during experimental orthodontic tooth movement (OTM). They also reported increased concentrations of M-CSF in GCF of the canine retraction side compared to the control side, indicating an increase in M-CSF–associated OTM allied to bone remodeling. The phenotypic expression of macrophages at the site of callus formation in mice is suggestive of their role in fracture repair.[27] Therefore, during the fracture healing

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phase, the presence of M-CSF seems necessary to support the proliferation and differentiation of osteoclasts, as suggested in the studies described above.

It is interesting to note that biomarkers such as M-CSF and RANKL, which have a role in bone remodeling, were found to rise later (1–3 weeks) than pro-inflammatory biomarkers such as IL-1β (4 hours after MSI insertion). Therefore, pro-inflammatory biomarkers are cytokines released by cells of the first line of defense, whereas bone remodeling cytokines (RANKL, M-CSF) are released in response to the early inflammation process. The osteoclastic activity by M-CSF is also known to increase in manifold fashion after stimulation by IL-1 and TNF (pro-inflammatory biomarkers), proportionately.[28]

This study highlighted the underlying biochemistry applicable to bone and tissue remodeling around MSIs, suggesting that they may be considered instrumental for the secondary stability of miniscrew implants. Limitations of the study included a subjective sample size, and evidence from the study should be supported by other bone remodeling biomarkers. Osteoclastic activity was not compared to the less stable or failing implants. Therefore, further extensive research into the biomarkers in PMICF, along with microbiological data and host response, is warranted. Further research and animal studies with immunohistochemistry can provide strong evidence of the underlying inflammatory processes, which may help avoid uncertain implant loosening.

CONCLUSIONS

- The maximum M-CSF activity around MSIs was found at about 3 weeks after miniscrew insertion, suggesting underlying bone remodeling after surgical injury.
- However, loading MSIs with orthodontic forces during the experimental period of 42 days postloading did not cause any significant surge in M-CSF levels.

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