Original Article

Levels of TGF- β 1 in peri-miniscrew implant crevicular fluidAvinash Kaur^a, Om Prakash Kharbanda^{b,*}, Moganty R. Rajeswari^c, Dinesh Kalyanasundaram^{a,d,**}^a Centre for Biomedical Engineering, Indian Institute of Technology Delhi, New Delhi, 110016, India^b Centre for Dental Education and Research, All India Institute of Medical Sciences, New Delhi, 110029, India^c Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, 110029, India^d Department of Biomedical Engineering, All India Institute of Medical Sciences, New Delhi, 110029, India

ARTICLE INFO

Keywords:

Miniscrews implants (MSIs)
 Peri-Miniscrew implant crevicular fluid (PMICF)
 TGF- β 1
 Inflammation

ABSTRACT

The peri-miniscrew implant crevicular fluid is analogous to gingival crevicular fluid, and its contents reflect the state of inflammation and health during the life of the miniscrews in the mouth. The stability of MSI is fundamental to its role as an anchorage. This study aimed to evaluate transforming growth factor-beta one (TGF- β 1) of the peri-miniscrew implant crevicular fluid (PMICF), on implant insertion, pre- and post-loading of MSIs to find a clue to their role in the stability of MSI. Fifty-two MSIs sites were placed in the mouths of 13 patients aged 12–26 years undergoing orthodontic treatment. PMICF was collected using micro-pipettes at T1 (day 0, 1 h after MSI implantation), T2 (day 1), T3/baseline (day 21, preloading of MSI), T4 (day 21, 1 h post loading), T5 (day 22, 1 day post loading), T6 (day 43, 3 weeks post loading). The levels of TGF- β 1 were estimated by enzyme-linked immunosorbent assay (ELISA). The data were subjected to statistical analysis. Of the 52 MSIs, 20 MSIs failed at T3. In the case of successful MSIs, the TGF- β 1 levels were found to monotonously decrease from T1 (~1400 pg/mL) until T3 (~700 pg/mL) and saturate thereafter. In the case of failed MSIs, the levels of TGF- β 1 at various time periods were approximately constant and of much lower value than corresponding time periods of successful MSIs. This study highlights the role of TGF- β 1 in bone metabolism around miniscrew reflecting the state of inflammation from 1 h post-implantation.

1. Introduction

Miniscrew implant (MSI) are temporary anchorage devices (TAD) used for orthodontic tooth movement (OTM).¹ MSIs are commercially available in various diameters and lengths. MSIs can be loaded immediately or within 3 weeks of insertion.^{2,3} An average success rate of 84% has been reported.⁴ TADs made of Ti6Al4V alloy are preferred due to its biocompatibility and desired strength. The success of MSIs is largely dependent on factors governing mechanical stability and biological retention.^{5–7}

The peri-miniscrew implant crevicular fluid (PMICF) is an inflammatory exudate that is secreted in the crevice between MSI and peri-implant (PI) tissue. The composition of PMICF is quite similar to gingival crevicular fluid (GCF) containing various inflammatory biomarkers including interleukins, growth factors, and other cytokines.⁸ The levels of these PMICF biomarkers vary with time as well as during different stages of implantation and mechanical loading.⁹ In the known literature, interleukins such as IL-1 β , IL-2, IL-6, IL-8, tumor necrosis

factor – alpha (TNF- α), receptor activator of nuclear factor kappa ligand (RANKL), chondroitin sulphate (CS) and osteoprotegerin (OPG) have been reported in the PMICF during OTM.^{9–13} However, transforming growth factor – beta 1 (TGF- β 1) levels have not been studied in PMICF during pre- and post-loading around MSIs.

TGF- β 1 is an osteoimmunological biomarker that is produced by T – regulatory cells (Treg), macrophages and osteoclasts.^{14,15} TGF- β 1 plays an important role in activation, proliferation, migration, and synthesis of extracellular matrix (ECM).¹⁶ Further, the biomarker also modulates proliferation and differentiation of osteoblasts by stimulating macrophage colony-stimulating factor (M-CSF).^{17,18} TGF- β 1 is involved in angiogenesis, immune suppression, apoptosis, and cell growth inhibition.¹⁹ The cellular source and function of TGF- β 1 is shown in Fig. 1. The concentration levels of TGF- β 1 have not been reported previously in PMICF. However, few studies on TGF- β 1 in GCF have been reported. Vikram et al. studied the levels of TGF- β 1 at three time points in GCF during pre-surgery, two-weeks and six-weeks post-surgery of chronic periodontitis (CP) patients. When compared to baseline values

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<https://doi.org/10.1016/j.jobcr.2020.03.002>

Received 30 December 2019; Received in revised form 19 February 2020; Accepted 3 March 2020

Available online 06 March 2020

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Fig. 1. Cellular source and function of TGF-β1.

(53.94 pg/mL), TGF-β1 levels were observed to be reduced at pre-surgery (24.08 pg/mL), at two weeks post-surgery (21.97 pg/mL) and at six weeks' post-surgery (14.78 pg/mL) indicating the important role of TGF-β1 in periodontal regeneration and wound healing.²⁰ Barbeiri et al. analyzed TGF-β1 levels in GCF during OTM on both the tension and compression sites at (i) 24 h (baseline) and at (ii) 7 days after the placement of orthodontic elastic separators. Increased levels of TGF-β1 levels were observed at the compression site (49.56 pg) than at tension site (30.74 pg) (after 7 days). This observation suggests that TGF-β1 has a vital role in bone resorption.¹⁸ No other literature on TGF-β1 of dental patients have been reported.

A short summary of the literature available on TGF-β1 levels in GCF is shown in Table 1 for reference. No studies on TGF-β1 levels have been reported in PMICF. Also, as discussed earlier, no literature has been reported on the concentration of TGF-β1 during implantation of MSIs, pre- and post-loading of MSIs. Therefore, this comprehensive study was undertaken with the aim to evaluate the changes in TGF-β1 levels in PMICF and its correlation with the clinical parameters (mobility and gingivitis) over the course of orthodontic treatment.

2. Materials and methods

2.1. Institute ethical approval

Ethical clearance was obtained from the Institute Ethics Committee (reference number IEC/NP-373 dated October 8, 2014). Written consent was obtained from each patient prior to the study. No history of systemic disease, hormonal imbalance or drug intake was noticed in the patients. All the patients had satisfactory periodontal health prior to the start of orthodontic treatment. Approximately, first three months were

spent for levelling and alignment of teeth of the patients before the start of the current study. MSIs were implanted as a routine procedure adopted at the medical institute for temporary anchorage for patients undergoing orthodontic treatment. No participants were recruited nor any MSI implanted merely for this study.

2.2. MSI placement and PMICF collection

MSIs were placed in 13 patients (mean age 19.3 ± 6.64 years) using hand screwdriver by the same orthodontist and treated with Roth prescription ($0.022'' \times 0.028''$). Four MSIs were placed in each patient at the following sites: right maxilla, left maxilla, left mandible, right mandible. The insertion torque was measured using Surgic pro[®] system (Nakanishi Inc., Japan). The insertion torque was in the range of 5–10 N-cm. PMICF samples were collected around the crevices of MSIs at six different time periods at T1 (day 0, 1 h after MSI implantation), T2 (day 1 after MSI implantation), T3/baseline (day 21, prior to mechanical loading of MSI), T4 (day 21, 1 h post loading), T5 (day 22, 1 day post loading), T6 (day 43, 3 weeks post loading). The study was conducted to follow a total of 52 MSIs implanted in the 13 volunteers. Twenty MSIs implanted in five patients created visible inflammation at T3 and were subsequently removed. These particular volunteers were subsequently not involved in the study any further.

The buccal interradicular bone in the attached gingiva between the second premolar and first molar was chosen as the site for MSI placement. Intraoral periapical radiograph (IOPAR) was performed before the placement of MSIs to check the availability of space between the roots of the second premolar and first molar. If sufficient space was not available, an upright band in the wire is introduced to upright the roots of the tooth. MSIs (8 mm long, 1.5 mm in diameter, bracket head type;

Table 1
Literature on TGF-β1 studied in GCF.

S.No	Reference	Biomarkers	Method of collection	Analysis method	No. of patients	Duration of study (days)	Time at which significant variation was achieved	Concentration		
								Pre-surgery	Post-surgery	Control
1	Vikram et al., ²⁷	TGF-β1 (GCF)	Micro-capillary pipettes	ELISA	18 sites	42	–	24.08 pg/mL	14.78 pg/mL	53.94 pg/mL
								Compression site	Tension site	
2	Barbeiri et al., ²⁵	TGF-β1 (GCF)	Paper strips	ELISA	10	7	–	49.56 pg	30.74 pg

Fig. 2. A, Assembly showing MSI placement; B, assembly showing mechanical loading via springs; C, swelling around MSI; D, migration of MSI.

Absoanchor, Dentos, Daegu, South Korea) were placed at the intended sites at 60° to the long axis of the teeth under local anesthesia as shown in Fig. 2. Miniscrews were directly loaded with 200 gm of force using nitinol close coil springs of 9 mm length for enmasse retraction. All patients were instructed and followed for oral hygiene such as frequent brushing using a soft brush and mouth rinsing with 15 mL of 0.2% chlorhexidine gluconate twice daily. No anti-inflammatory drug was prescribed prior to and after MSI placement.

2.3. Schedule of sample collection and clinical examination

Approximately about 1 μ L of PMICF was collected around the MSIs from every volunteer at various time intervals using a micro-capillary pipette (Sigma Aldrich, Bangalore, India). Sufficient care was taken to avoid pipetting of GCF that is generally around the gingival sulcus. The method of PMICF extraction is described elsewhere in detail.²¹ PMICF were collected at six time periods: T1 to T6. PMICF sample T1 was taken 1-h post-MSI implantation to allow for the cessation of bleeding. However, PMICF samples that were contaminated with blood were discarded. Isolation of the MSI site was performed with a cheek retractor, cotton rolls, and a gentle air spray. MSI implantation and PMICF sample collection were carried out in the morning hours between 9 a.m. and 11 a.m. Extreme care was taken not to cause any slightest harm or injury during the sample collection. The samples thus obtained were diluted to 1:200 PBS buffer (phosphate buffered saline, pH 7.0), centrifuged at 2000 RPM for 1 min and stored at -80°C until assayed for ELISA testing of TGF- β 1 levels.

2.4. Successful versus failed MSIs

Presence of any signs of peri-MSI inflammation (redness, swelling, pain) was detected clinically. Manual mobility was evaluated using the blunt ends of the mirror handles. Also, the mobility was assessed using Periotest™ (Bensheim, Germany). The periotest with values of 0–9 indicate good stability (mechanical) while values higher than 9 indicate clinical failure.^{22,23} Loe and Silness index were also evaluated to determine gingival status. A manual mobility between 1 and 3 combined with periotest value more than 9 and Modified Index of Loe and Silness value of 3 were considered as failed MSIs (Table 2).

Table 2

Periotest scale for evaluation of mobility of MSI.

Group	Clinical signs	Mobility		Loe and Silness index
		Manual	Periotest value	
Successful MSIs	No redness, swelling, pain	No detectable mobility (0)	< 9	0–1
Failed MSIs	Redness, swelling, pain present	Detectable mobility (1–3)	> 9	3

2.5. Quantification of TGF- β 1 levels in PMICF

TGF- β 1 concentrations in PMICF were estimated using ELISA® kit (Cloud clone corp., Houston, USA). Optical density was measured at 450 nm using Omega Microplate Reader™ (BMG Labtech, Ortenberg, Germany). The quantification of TGF- β 1 was determined using standard curves.

2.6. Statistical analysis

The overall p value was calculated using Friedman test. The statistical significance within each of the groups (i.e. successful or failed MSIs) was evaluated by Wilcoxon signed-rank non-parametric test. For the comparison between groups i.e. successful versus failed groups, Wilcoxon rank-sum test was used. Results were considered statistically significant for $p < 0.05$ for all the statistical tests. The data were analyzed using SPSS® version 20 software and represented in mean, median, maximum and minimum.

3. Results

Thirty-two successful MSIs displayed no signs of clinical mobility. The periotest mobility values ranged between 0 and 9 indicated all 32 MSIs to be mechanically stable with no detectable manual mobility and periotest value of < 9. The modified Loe and Silness index for the successful MSI ranged between 0 and 1, suggesting normal gingiva status (Table 2). For the failed MSI, the manual mobility ranged between 1 and 3, the periotest index was higher than 9 and the modified Loe and Silness index was 3, therefore indicating clinical failure of the MSIs. The concentrations of TGF- β 1 at each of the four sites were

Fig. 3. TGF- β 1 levels (pg/mL) in PMICF at different time intervals (combined maxilla and mandible): [T1 (day 0, 1 h after MSI implantation), T2 (day 1, 1 day after MSI implantation), T3 (day 21 after 3 weeks', pre-loading), T4 (day 21, 1 h post loading), T5 (day 22, 1 day post loading), T6 (day 43, 3 weeks' post loading)] (a) successful MSIs (b) failed MSIs. The boxes show the values from 25th to 75th percentile. The middle line indicates the median values. The vertical line extends from minimal to maximal values, excluding outliers pointed as small dark circles.

analyzed in PMICF samples of each patient. Among the four quadrants, the values were observed to be equal to each other with no statistical distinction. TGF- β 1 levels of thirteen patients including eight patients with successful MSIs and five patients with failed MSIs, observed at various time periods in all the four quadrants are shown in Fig. 3.

TGF- β 1 levels for successful MSIs at T1 (1378.91 pg/mL) and T2 (834.37 pg/mL) were significantly higher than the baseline T3 (708.10 pg/mL) as shown in Fig. 3. At T4, T5 and T6 the levels were 704.71 pg/mL, 712.60 pg/mL and 687.10 pg/mL respectively. The overall p value was less than 0.05 and hence was statistically significant (Table 3). In the case of failed MSIs, the TGF- β 1 level at T1 was 305.69 pg/mL. The value was significantly lesser than T1 of successful MSI. In comparison with the baseline T3 (280.69 pg/mL) of failed MSIs, the T1 values are merely marginally higher than the baseline (T3). From T1, the level increased to 436.56 pg/mL at T2 and was statistically significant ($p = 0.04$) compared to baseline (T3). The overall p value was 0.02 (Table 3) indicating statistical significance. In the statistical comparison between successful versus failed groups, the p -value at T1, T2, and T3 was 0 in all the three cases, specifying statistical significance. The levels of TGF- β 1 measured in GCF before T1 (control) was 483.10 pg/mL which was in between failed and successful MSIs at all T1 to T6. The value of T1 of successful MSI (1378.91 pg/mL) was higher than four times than that of T1 value of failed MSIs (305.69 pg/mL).

4. Discussion

MSIs are widely used for temporary anchorage in contemporary clinical practice.¹ The implantation of MSIs leads to inflammation thereby causing the release of both pro- and anti-inflammatory osteoimmunological biomarkers. The biomarkers including IL-1, IL-12, IL-18, and TNF etc. are produced by T-helper cells (Th cells), macrophages and other cells types.^{24,25} The above-cited biomarkers are pro-inflammatory cytokines and are involved in the upregulation of vascular permeability, chemoattraction of circulating leukocyte, proteolysis by causing fever, inflammation, tissue destruction and in some cases even shock and death.²⁶ Hamamci et al. evaluated the levels of IL-2, 6 and 8

Table 3
Statistical analysis of ELISA results for TGF- β 1 levels (pg/mL).

Time period	Successful group		Wilcoxon signed rank test		Failed group		Wilcoxon signed rank test		Wilcoxon rank sum test	
	Mean $\pm$ SD	Median (minimum, maximum)	Median (minimum, maximum)	p value (comparison against T3 within successful group)	Mean $\pm$ SD	Median (minimum, maximum)	p value (comparison against T3 within failed group)	p value (between successful vs failed groups)		
T1	1425.64 $\pm$ 132.71	1378.91 (1293.37, 1698.23)	305.69 (295.35, 366.45)	0.01 ^b	314.91 $\pm$ 29.51	305.69 (295.35, 366.45)	0.22	0.00 ^c		
T2	840.40 $\pm$ 52.00	834.37 (766.37, 910.45)	436.56 (398.25, 476.64)	0.01 ^b	442.67 $\pm$ 31.54	436.56 (398.25, 476.64)	0.04	0.00 ^c		
T3	707.96 $\pm$ 32.48	708.10 (650.87, 750.47)	280.69 (250.32, 350.24)	NA	291.36 $\pm$ 37.13	280.69 (250.32, 350.24)	NA	0.00 ^c		
T4	712.81 $\pm$ 43.56	704.71 (650.89, 780.45)	NA	0.48	NA	NA	NA	NA		
T5	724.22 $\pm$ 42.41	712.60 (680.43, 790.59)	NA	0.40	NA	NA	NA	NA		
T6	686.79 $\pm$ 36.80	687.10 (636.39, 745.36)	NA	0.40	NA	NA	NA	NA		
p value (using Friedman test)	0.00 ^a				0.02 ^a					

^a $p < 0.05$ is considered significant for Friedman test.

^b $p < 0.05$ is considered significant for Wilcoxon signed rank test.

^c $p < 0.05$ is considered significant for Wilcoxon rank sum test.

in GCF and PMICF at 2 weeks after MSI implantation. IL-2 and 8 were observed to be high after 24 h of loading while no change was observed in IL-6.²⁷ Monga et al. observed an increase in the levels of IL-1 β in PMICF on MSI implantation and during immediate loading, thereby indicating the important role of IL-1 β in inflammation.²⁸

The biomarker of interest in the current work is TGF- β 1. TGF- β 1 is an anti-inflammatory cytokine that suppresses the activity of pro-inflammatory cytokines during tissue destruction.²⁹ TGF- β 1 also plays a role in boosting periodontal regeneration³⁰ and is regarded as a bone formation marker involved in osteoblastic activities.³¹ TGF- β 1 increases the production of OPG and RANKL through pro-osteoclastic cells.^{15,32} It plays important role in the chemotactic and the mitogenic activity of gingival and periodontal ligament fibroblasts as well as during the upregulation of ECM components including collagen, fibronectin, tenascin and proteoglycans.³³ However, the inflammatory response is an exceedingly complex interplay of cytokines whose exact role of pro-inflammatory or anti-inflammatory is dependent upon multiple parameters.³⁴ TGF- β 1 levels were found to be reduced during pre-surgery, two and six weeks post-surgery in GCF of chronic periodontitis patients.²⁰ This study was not in accordance with the findings of Kuru et al. who reported an increase in TGF- β 1 levels up to two weeks following surgery in GCF.³⁰ In our present study, TGF- β 1 levels were found to be significantly higher at T1 (1 h), T2 (day 1) and T3 (day 21/3 weeks) suggesting that it plays important role in bone metabolism. The high concentration of TGF- β 1 at T1 in successful versus failed MSIs indicate the potential of the cytokine for inflammation. This is the prime contribution of the current work. In future, a microdevice to evaluate the TGF- β 1 level can be helpful for early detection of the potential failure.

5. Conclusion

There is a significant difference in the levels of TGF- β 1 between the failed and successful implants in patients as early as 1 h post implantation. This observation strongly implies that TGF- β 1 can be used to predict the successful outcome, which bears a huge clinical relevance in patients undergoing orthodontic treatment. The fact that currently known inflammatory markers assess only late inflammation is not very helpful in predicting the outcome.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Abbreviations

TGF- β 1	Transforming growth factor-beta one
PMICF	Peri-miniscrew implant crevicular fluid
MSIs	Miniscrews implants
TAD	Temporary anchorage devices
OTM	Orthodontic tooth movement
PI	Peri-implant tissue
GCF	Gingival crevicular fluid
TNF- α	Tumor necrosis factor – alpha
RANKL	Receptor activator of nuclear factor kappa ligand;
CS	Chondroitin sulphate
OPG	Osteoprotegerin
ECM	Extracellular matrix
M-CSF	Macrophage colony-stimulating factor
CP	Chronic periodontitis

Ethics approval and consent to participate

Ethical clearance was obtained from Institute Ethics Committee (reference number IEC/NP-373 dated October 8, 2014) and written informed consent was obtained from all participants for this research.

Consent for publication

The authors hereby consent for publication.

Availability of data and material

All the relevant data are provided in the manuscript. For any queries, the corresponding author may be contacted via email.

Author's contributions

OPK, MRR and DK conceived the idea; AK and OPK collected the samples; AK and MRR analyzed the samples; AK prepared the initial draft; OPK, MRR and DK edited the manuscript.

Funding

The authors would like to acknowledge the funding agencies for their continued support: All India Institute of Medical Sciences (Intramural grant/2014), BIRAC, Department of Biotechnology (BIRAC/BT/AIR0275/PACE-12/17) and Indian Council for Medical Research(CARE/NCTFW/2018).

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