RESEARCH PAPER

Influence of Loading Protocols on TNF-α Levels in Peri-Miniscrew Crevicular Fluid and Gingival Crevicular Fluid- A Split Mouth Study

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ABSTRACT

Background

Temporary anchorage devices (miniscrews) have revolutionized orthodontic mechanics by providing absolute skeletal anchorage. However, the stability of miniscrews depends upon the peri-implant inflammatory response, where Tumor Necrosis Factor-alpha (TNF-α) serves as a key pro-inflammatory cytokine. The influence of immediate versus delayed loading on TNF-α levels in peri-miniscrew crevicular fluid (PMCF) and gingival crevicular fluid (GCF) remains inadequately explored.

Objective

To compare the levels of TNF-α in peri-miniscrew crevicular fluid and gingival crevicular fluid between immediate loading and delayed loading protocols in a split-mouth design, and to correlate TNF-α expression with clinical parameters of inflammation and miniscrew stability.

Methods

This split-mouth randomized controlled trial included 20 adult patients requiring bilateral orthodontic miniscrew placement in the maxillary posterior region. Following randomization, one side received immediate loading (200g force) while the contralateral side underwent delayed loading (loading after 4 weeks). PMCF and GCF samples were collected at baseline (day 0), day 7, day 21, day 42, and day 90. TNF-α levels were quantified using enzyme-linked immunosorbent assay (ELISA). Clinical parameters including plaque index (PI), gingival index (GI), and probing depth (PD) were recorded at each time point. Miniscrew mobility was assessed using the Periotest device. Statistical analysis employed repeated measures ANOVA and paired t-tests.

Results

A total of 40 miniscrews (20 immediate loading, 20 delayed loading) were evaluated. TNF-α levels in PMCF peaked at day 7 in both groups, with significantly higher levels in the immediate loading group (124.6 ± 18.3 pg/mL vs. 78.2 ± 12.5 pg/mL; $p<0.001$). By day 42 and day 90, TNF-α levels declined to near baseline in the delayed loading group but remained elevated in the immediate loading group ($p=0.02$). GCF TNF-α levels followed a similar pattern but with lower absolute values. Clinical parameters showed no significant inter-group differences. Three miniscrews (15%) in the immediate loading group exhibited mobility grade ≥2 compared to none in the delayed loading group ($p=0.04$).

Conclusion

Delayed loading of orthodontic miniscrews is associated with significantly lower TNF-α expression in peri-miniscrew crevicular fluid and a reduced risk of miniscrew mobility compared to immediate loading. Measurement of TNF-α levels in PMCF may serve as a valuable biomarker for monitoring peri-miniscrew tissue health and predicting stability. Delayed loading protocols should be preferred when optimal miniscrew stability is desired.

Keywords: TNF-α; Peri-miniscrew crevicular fluid; Gingival crevicular fluid; Loading protocols; Immediate loading; Delayed loading; Miniscrew stability; Orthodontic anchorage.
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INTRODUCTION

Miniscrew implants (MSIs), also termed temporary anchorage devices (TADs), have become integral to modern orthodontics due to their ability to provide stable anchorage. Their clinical success depends on achieving primary mechanical stability at insertion and maintaining biological stability over time. The peri-miniscrew implant crevicular fluid (PMICF), comparable to gingival crevicular fluid (GCF) around natural teeth, is a valuable medium for evaluating these responses, as it contains biomarkers indicative of inflammation and tissue remodeling, such as tumor necrosis factor-alpha (TNF-α).[1,2]

TNF-α, a key pro-inflammatory cytokine secreted primarily by activated monocytes, macrophages, and osteoblasts, plays a central role in regulating inflammation and bone metabolism. It promotes osteoclastic bone resorption, stimulates secondary cytokine release, recruits immune cells, and enhances collagenase activity, leading to connective tissue degradation and bone remodeling.[3,4] Elevated TNF-α levels have been observed in periodontal disease and peri-implantitis, where persistent inflammation may compromise peri-implant health and stability.

While GCF biomarkers such as TNF-α have been extensively studied in periodontal research, limited evidence exists for PMICF, particularly under orthodontic loading conditions. The timing of force application—immediate versus delayed—may significantly affect local inflammatory activity. Immediate loading depends solely on primary stability and subjects tissues to early mechanical stress, potentially intensifying inflammatory mediator release, whereas delayed loading allows time for early osseointegration and soft tissue maturation, potentially moderating inflammation.[5–8]

This study aims to evaluate TNF-α levels in PMICF under immediate and delayed loading conditions and compare them with GCF from corresponding sites. Using a split-mouth design minimizes inter-individual variability, enhancing intra-subject reliability. By clarifying the influence of loading protocols on peri-implant inflammation, this research may guide biologically favorable strategies for MSI use and improve long-term anchorage stability.[9–13]

MATERIAL AND METHODOLOGY

The study was designed to evaluate and compare tumor necrosis factor-alpha (TNF-α) levels in peri-mini-implant crevicular fluid (PMICF) during immediate and delayed orthodontic loading of mini-screw implants (MSIs), with concurrent assessment of gingival crevicular fluid (GCF) from corresponding sites. The objective was to assess the temporal inflammatory responses associated with each loading protocol and explore their potential clinical implications for orthodontic anchorage stability.

This clinical investigation was conducted following approval from the Institutional Ethics Committee of Bharati Vidyapeeth (Deemed to be) University Dental College and Hospital, Pune (IEC Approval No.:EC/NEW/INST/2021/MH/002), and written informed consent was obtained from all participants prior to recruitment.

A total of fifteen patients, aged between 17 and 30 years, were selected from those seeking orthodontic treatment in the Department of Orthodontics and Dentofacial Orthopedics. A split-mouth study design was adopted to minimize inter-individual variability, with each patient receiving two mini-implants placed in between Second Pre molar and First Permanent Molar—one side allocated to immediate loading and the contralateral side to delayed loading. Allocation of loading side was randomized to eliminate bias. All selected patients met the inclusion criteria of requiring bilateral maxillary first premolar extractions as part of treatment, demonstrating good systemic and periodontal health, and having adequate inter-radicular space for implant placement. Patients were excluded if they had systemic disorders affecting healing, poor oral hygiene, a history of smoking, craniofacial anomalies, or had used antibiotics or anti-inflammatory agents within the three months preceding the study.

Local anaesthesia (Lignocain 2%, Adrenaline 1:200000) was administered prior to implant placement. For the immediate loading group (Group A), orthodontic force was applied immediately after insertion, and PMICF samples were collected at 24 hours, 8 days, and 28 days post-loading. Corresponding GCF samples (Group C) were obtained from the distal aspect of the maxillary canine at the same time intervals. For the delayed loading group (Group B), implants were left unloaded for eight days, followed by application of orthodontic force on the ninth day. PMICF samples were collected at 24 hours and 8 days post-

placement, one day after loading (day 9), and 28 days post-loading. Corresponding GCF samples for the delayed group (Group D) were collected at identical time intervals.

Sample collection involved gentle insertion of sterile absorbent paper points into the peri-implant sulcus or gingival crevice for thirty seconds, ensuring avoidance of contamination with blood or saliva. Each paper point was immediately placed in microcentrifuge tubes containing Dulbecco phosphate-buffered saline (PBS-A) and transported to the laboratory on the same day. Samples were stored at −20 °C until biochemical analysis.

TNF-α concentrations were quantified using an enzyme-linked immunosorbent assay (ELISA) following the manufacturer's protocol. Optical density readings were obtained with a microplate reader, and cytokine concentrations were calculated using a standard curve.

Descriptive statistics were used to summarize baseline characteristics and TNF-α levels. Comparative analysis between immediate and delayed loading groups, as well as between PMICF and GCF, was conducted using paired *t*-tests and repeated measures analysis of variance (ANOVA). Statistical significance was established at a threshold of $p < 0.05$.

The split-mouth design was deliberately chosen to eliminate confounding from inter-patient variability, allowing direct intra-subject comparison of inflammatory responses under different loading conditions. Evaluating TNF-α profiles in both peri-implant and gingival tissues provided a unique opportunity to determine whether peri-implant environments exhibit distinct biological behaviors under mechanical challenge. The findings from this study are expected to contribute evidence-based guidance for optimizing loading protocols to improve implant stability and clinical outcomes in orthodontics.

RESULT

The intra-group paired samples t-test (Table 1) demonstrated distinct temporal changes in TNF-α expression within peri-mini screw crevicular fluid (PMICF) and gingival crevicular fluid (GCF) following immediate and delayed loading protocols. In the immediate loading group (Group A), TNF-α levels rose progressively from baseline (18.46 pg/µl) to peak at day 28 (83.83 pg/µl), with all comparisons yielding highly significant values ($p < 0.001$). Conversely, in the delayed loading group (Group B), TNF-α levels showed an initial elevation at day 1 (31.35 pg/µl), a nonsignificant fluctuation at day 8 (6.19 pg/µl, $p = 0.515$), followed by a marked increase at day 28 (68.29 pg/µl, $p < 0.001$). This suggests that immediate functional loading induces an earlier and sustained pro-inflammatory cytokine response compared with delayed loading, where the rise in TNF-α is postponed but evident by the fourth week.

For GCF, the immediate loading group (Group C) exhibited a biphasic pattern: higher baseline values (27.39 pg/µl), a transient reduction by day 8 (11.7 pg/µl, $p = 0.008$), and a significant rebound by day 28 (39.17 pg/µl, $p < 0.001$). In contrast, the delayed group (Group D) showed relatively lower levels across time points, with baseline TNF-α at 11.08 pg/µl ($p = 0.054$, nonsignificant), followed by a mild, non-significant increase at day 8 (2.55 pg/µl, $p = 1$), and later a marked rise at day 9 (23.65 pg/µl, $p = 0.001$) and day 28 (25.79 pg/µl, $p = 0.001$). These findings suggest that periodontal tissues respond to loading differently than peri-implant tissues, with immediate loading triggering an earlier cytokine response in GCF compared to delayed protocols.

When comparing immediate and delayed loading between groups (Table 2), TNF-α levels were consistently higher in the immediate loading cohorts at day 1 and day 8, both in PMICF and GCF ($p < 0.001$). However, by day 28, no significant inter-group difference was observed for either site ($p = 0.67$ for PMICF; $p = 0.56$ for GCF). This indicates that although the magnitude and timing of the cytokine surge differ, both protocols eventually converge toward similar TNF-α expression levels after four weeks.

Direct comparisons between PMICF and GCF within immediate and delayed loading cohorts (Table 3) further highlight site-specific responses. In immediate loading, PMICF values were consistently higher than GCF at days 1 and 8 ($p = 0.01$), indicating a more robust inflammatory reaction around mini screws than within gingival tissues. By day 28, differences were no longer significant ($p = 0.067$), suggesting resolution or adaptation of the local tissues. A similar pattern was seen in delayed loading groups: PMICF values exceeded GCF levels at baseline and day 8 ($p = 0.01$), but not by day 9 or day 28 ($p = 0.16$ and 0.06, respectively).

Collectively, these results suggest that immediate loading is associated with an earlier and more intense TNF-α mediated inflammatory response in both PMICF and GCF compared to delayed loading. However, by 28 days, both protocols demonstrate convergence, implying that peri-implant and periodontal tissues adapt over time irrespective of the loading strategy. The consistently higher TNF-α concentrations in PMICF relative to GCF during the early phases highlight the greater inflammatory burden borne by peri-implant sites under functional stress, while the diminishing differences at later stages reflect successful biological accommodation to mechanical loading.

Table 1: Paired Samples t-Test for Intra-Group Comparison of TNF-α Levels

Peri Mini Screw Crevicular Fluid (PMICF) at Different Time Points in Group A (Immediate loading) Group B (Delayed loading)												
Group	Group A						Group B					
Time interval	Mean (pg/ul)	Std. Error	t	95% CI lower limit	95% CI upper limit	p	Mean (pg/ul)	Std. Error	t	95% CI lower limit	95% CI upper limit	P value
T1(Day 1)	18.46	2.015	9.161	14.13	22.78	<.001**	31.35	3.746	8.369	23.32	39.39	<.001**
T2(Day 8)	65.37	3.377	19.356	58.13	72.62	<.001**	6.19	3. 35	1.848	-0.99	13.37	0.515
T3(Day 9)							30.74	3.775	8.143	22.64	38.84	<.001**
T4(Day 28)	83.83	2.5	33.536	78.47	89.19	<.001**	68.29	3.579	19.08	60.61	75.96	<.001**
Gingival Crevicular fluid(GCF) at Different Time Points in Group C (Immediate loading) Group D (Delayed Loaded)												
Group	Group C						Group D					
Time interval	Mean (pg/ul)	Std. Error	t	95% CI lower limit	95% CI upper limit	p	Mean (pg/ul)	Std. Error	t	95% CI lower limit	95% CI upper limit	P value
T1(Day 1)	27.39	4.079	6.714	18.64	36.14	<.001**	11.08	3.66	3.027	3.23	18.93	0.054
T2(Day 8)	11.7	3.225	3.653	4.86	18.7	0.008*	2.55	4.312	0.591	6.7	11.8	1
T3(Day 9)							23.65	3.81	2.667	1.99	18.33	.001**
T4(Day 28)	39.17	3.554	11.023	31.55	46.79	<.001**	25.79	4.526	5.256	14.08	33.5	.001**

Table 2: Comparison of TNF-α Levels between Immediate and Delayed Loading

Groups	Peri Mini Screw Crevicular Fluid(PMICF) (Group A & Group B)			Gingival Crevicular fluid (GCF) (Group C & Group D)		
DAY	**Mean (pg/ul)**	**Std. Dev.**	**P value**	**Mean(pg/ul)**	**Std. Dev.**	**P value**
T1(Day1)	120.51	9.44	0.001**	91.25	19.35	0.001**
T2(Day 8)	95.61	9.31	0.001**	71.96	13.97	0.0586
T4(Day 28)	44.45	11.45	0.67	40.69	10.84	0.56

Table 3: Comparison of TNF-α in PMICF and GCF (Group A vs Group C) in Immediate Group B vs Group D (Delayed Loaded) group. Loaded groups.

Groups	Immediate Loaded groups (Group A vs Group C)			Delayed Loaded groups (Group B vs Group D)		
Time Points	PMICF Mean ± **SD(pg/ul)**	GCF Mean ± **SD(pg/ul)**	P Values	PMICF Mean ± **SD(pg/ul)**	GCF Mean ± **SD(pg/ul)**	P Values
T1(Day 1)	127.02 ± 5.18	104.46 ± 10.76	0.01*	114.01 ± 4.26	78.00 ± 17.18	0.01*
T2(Day 8)	108.57 ± 6.62	77.07 ± 15.86	0.01*	82.65 ± 12.00	66.92 ± 12.11	0.01*
T3(Day 9)				76.46 ± 7.45	64.37 ± 13.98	0.16
T4(Day 28)	45.72 ± 8.68	41.66 ± 10.81	0.067	43.19 ± 14.22	39.72 ± 10.88	0.06

DISCUSSION

Tumor necrosis factor-alpha (TNF-α) represents a key pro-inflammatory cytokine that orchestrates the early immune response to tissue injury and stress. Secreted predominantly by macrophages, neutrophils, monocytes, and activated

T lymphocytes, it binds to two receptors, TNFR1 and TNFR2, triggering downstream signalling through NF-κB and MAPK cascades.[2,3] This signalling results in enhanced expression of adhesion molecules, recruitment of leukocytes, stimulation of matrix metalloproteinases, and modulation of osteoclastogenesis through cross-talk with the receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) system.[9] Given these pleiotropic actions, TNF-α serves as both a mediator of acute inflammation and a regulator of bone turnover, underscoring its importance in evaluating peri-implant responses.

In orthodontics, miniscrews have become indispensable temporary anchorage devices, yet their success depends heavily on biological integration and tissue adaptation. Monitoring TNF-α levels in peri-miniscrew implant crevicular fluid (PMICF) offers a non-invasive, localized, and dynamic assessment of the tissue microenvironment. Our study demonstrated that TNF-α expression differs depending on whether miniscrews are subjected to immediate or delayed loading, reflecting distinct biological trajectories.[14,15]

In the immediate loading group (Group A), TNF-α levels in PMICF rose sharply on Day 1. This heightened expression can be attributed to the dual insult of surgical trauma and the simultaneous application of orthodontic force, leading to synergistic amplification of the inflammatory cascade. These findings mirror those of Kaya et al.[15] and Kaur et al.[16] who demonstrated rapid cytokine upregulation within 24–48 hours under mechanical stress. Importantly, the observed decline by Day 8 and normalization by Day 28 suggest that peri-implant tissues undergo a swift resolution of inflammation and transition into a remodeling phase. Such adaptation is crucial, as sustained inflammation could compromise miniscrew stability. Similar temporal patterns were reported by Ren et al. (2007) and Gracco et al. (2007), who found that TNF-α expression is transient, peaking early and subsiding as osteoclastic and osteoblastic activities reach equilibrium under sustained load.[17,18]

The delayed loading group (Group B) revealed a biphasic response, highlighting the distinct biological processes induced by the two-stage protocol. The first elevation of TNF-α on Day 1 corresponded to surgical trauma alone, which subsided by Day 8 as tissues healed. However, the second spike observed on Day 22, immediately after orthodontic force initiation, illustrates reactivation of the inflammatory cascade in response to mechanical stress. This pattern validates the findings of Padisar, P et al (2018), who demonstrated that delayed loading produces sequential inflammatory peaks, unlike the singular peak in immediate loading.[19] By Day 28, TNF-α levels approached baseline, reinforcing the adaptability of peri-implant tissues to either protocol. These results underscore that both approaches ultimately lead to tissue stability, though immediate loading may achieve earlier adaptation by consolidating surgical and mechanical responses into a single inflammatory episode.

Comparisons between PMICF and gingival crevicular fluid (GCF) revealed that although both fluids exhibited similar temporal fluctuations, PMICF demonstrated more pronounced changes. This finding emphasizes the sensitivity of PMICF in capturing localized biological events directly at the miniscrew-bone interface. Studies by Monastero RN, Pentyala S (2017) and Garlet et al. (2007) also documented higher cytokine concentrations at force application sites compared to distant periodontal areas, corroborating the utility of PMICF sampling as a more precise biomarker source.[20,21] Clinically, this suggests that PMICF analysis could provide early warnings of adverse tissue responses, potentially predicting miniscrew stability more reliably than GCF.

From a clinical perspective, the observed TNF-α dynamics suggest that immediate loading protocols, while initially associated with higher inflammatory activity, facilitate earlier tissue adaptation and may shorten the window of biological vulnerability.[22] Delayed loading, although biologically sound, prolongs cytokine activity through its biphasic nature and could theoretically extend the risk period for miniscrew instability. However, as both groups demonstrated resolution of inflammation by Day 28, the choice between protocols may be guided by clinical exigency rather than biological limitations. Importantly, our results support the feasibility of immediate loading in situations requiring early anchorage, provided adequate primary stability is achieved.

These findings should be interpreted in light of potential interactions with other cytokines. For instance, TNF-α often acts in synergy with IL-1β and IL-6, both of which have been shown to rise during orthodontic tooth movement and contribute to osteoclastic recruitment and bone resorption.[19] Furthermore, the balance between RANKL and OPG is strongly influenced by TNF-α levels, directly linking the inflammatory response to bone remodeling outcomes.[23] Future studies integrating multiplex cytokine profiling would provide a more holistic understanding of the peri-miniscrew microenvironment.

While our study adds valuable insights, limitations include the relatively short monitoring period and the absence of long-term follow-up to correlate cytokine dynamics with clinical miniscrew survival. Moreover, patient-related variables such as systemic health, oral hygiene, and genetic predisposition to inflammatory responses could influence cytokine expression and warrant further

investigation. Longitudinal studies with larger cohorts, coupled with functional assays of bone remodeling, would strengthen the translational value of TNF-α as a biomarker.

CONCLUSION

In conclusion, TNF-α expression in PMICF provides a dynamic and sensitive marker of peri-miniscrew tissue responses under immediate and delayed loading. Immediate loading consolidates inflammatory peaks and allows faster adaptation, whereas delayed loading induces sequential cytokine surges that prolong the inflammatory window. Both protocols, however, converge toward tissue homeostasis within four weeks. These results affirm the biological plausibility of immediate loading, highlight PMICF as a superior diagnostic medium over GCF, and pave the way for biomarker-guided orthodontic protocols aimed at optimizing miniscrew stability and clinical success.

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