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# Evaluation of the Levels of Interleukin 8 and Interleukin 4 around Miniscrew Implants During Orthodontic Tooth Movement: A Clinical Study

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## ABSTRACT

**Background:** The evaluation of cytokines in the peri-miniscrew implant crevicular fluid (PMICF) has been proposed as a non-invasive means of monitoring the healthy or diseased status of peri-implant tissues. The objective of this study was to evaluate the levels of interleukin 8 (IL-8) and interleukin 4 (IL-4) around miniscrew implants during orthodontic tooth movement and to draw clinical inferences from the same.

**Methodology:** The study was done in retraction stage around 22 healthy titanium miniscrew implants. The samples were collected over a month time period after force application on miniscrew implants according to the following schedule: T1, T2 and T3 at 1 hour, 7th day and 21st day respectively. The PMICF samples were put through ELISA test for determining the concentration of IL-8 and IL-4. The data was analyzed using descriptive and inferential statistics that included paired t test and independent t test.

**Result and interpretation:** It was observed that in healthy miniscrew implants the IL- 8 levels increased from one hour to 7th day due to perimucositis and slightly decreased or remained the same from 7th to 21st day indicating that the progress of inflammation has regressed. The IL-4 levels decreased from one hour to 7th day due to perimucositis. It decreased from 7th to 21st day. This could be attributed to the persistence of inflammation through 21st day.

**Conclusion:** These findings demonstrated that the levels of IL-8 and IL- 4 in PMICF could be considered in detecting the stability of miniscrew implants.

**Keywords:** Miniscrew implant, Peri-miniscrew implant crevicular fluid, Interleukin 8, Interleukin 4.

## INTRODUCTION

Anchorage is a critical issue during orthodontic treatment. If inadequate it can be the most limiting factor, no matter which technique or philosophy the clinician follows especially when treating adults. The first successful attempt to move teeth against a stationary screw was published in 1969 by Linkow. But the routine application of dental implants for orthodontic purposes in humans began 20 years later.[1]

Many disadvantages of dental implants became apparent during this period. To overcome that Kanomi in 1997 and Costa et al. in 1998 introduced miniscrew implants as temporary anchorage

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devices (TADs). Their decreased diameter facilitates insertion in almost all sites of the jaws with no flap surgery required. Osseointegration does not usually occur thereby providing only temporary stationary anchorage without a 6-month waiting period. Immediate loading of TAD apparently decreases total treatment time.[1] From this point onwards, the single most important implant used during orthodontic treatment for an anchorage is the miniscrew implant. It is routinely used to anchor retraction of the anterior segment, mesiodistal movement of the posterior teeth, asymmetrical tooth movement, intrusive mechanics and orthopaedic corrections.

The miniscrew implants have an average survival rate of 84% (range 57–95.3%) which is largely dependent on factors governing primary and secondary stability. The primary stability pertains to the mechanical holding of miniscrew implant in the bone, while the secondary stability relates to biological retention. Contemporary research has focused on the biological seal between the implant and host tissue, more so on its surrounding peri-implant oral epithelium.[1]

The occurrence of screw failures has required ongoing investigation of potential risk factors. Since peri-implantitis turned out to be the main cause of screw loosening, assessment of factors involved in such inflammation has become crucial to increase TAD stability in orthodontics.[1] Peri-implantitis is progressive peri-implant bone loss in conjunction with soft tissue inflammatory lesion and accounts for about 30% of miniscrew failures.[3,4] Initially, peri-mucositis (a reversible inflammation of the soft tissues surrounding the miniscrew) occurs, which if left untreated may progress to peri-implantitis.[3,5] Peri-implantitis is clinically characterized by increased probing depth, pain, and/or radiographic bone loss, which may cause implant failure.

Peri-miniscrew implant crevicular fluid (PMCIF) is an osmotically mediated inflammatory exudate originating from the vessels of the gingival plexus. Its composition is similar to that of the gingival crevicular fluid (GCF) containing host-derived enzymes and their inhibitors, inflammatory mediators, host response modifiers and tissue breakdown products.[6] Analysis of PMICF offers a non-invasive means of studying host response in peri-miniscrew disease and may provide an early indication of patients at risk for active disease.[7]

Cytokines are involved in initiating, amplifying, perpetuating and resolving inflammatory responses in periodontal and peri-implant tissues.[8]Cytokines are classified as pro-inflammatory and anti-inflammatory. Pro-inflammatory cytokines include TNF, IL-1, IL-2, IL-6 and IL-8. These induce vascular dilation through increased permeability and enhanced inflammatory response. Anti-inflammatory cytokines include IL-4, IL-10, IL-13, IFN-alpha and transforming growth factor-beta which are involved in the reduction of inflammatory reactions.[8]

IL-8 plays a key role in recruiting and activating neutrophils during inflammation. It is important for regulating alveolar bone resorption during tooth movement by acting at an early stage in the inflammatory response. IL-8 is secreted mainly by monocytes, macrophage, epithelial cells and endothelial cells with a role in neutrophil recruitment and degranulation during inflammation.[8]

Interleukin-4 (IL-4) is a pleiotropic cytokine secreted by activated T lymphocytes, mast cells, eosinophils, and basophils. It is a key regulator in humoral and adaptive immunity that regulates the function of lymphocytes and macrophages.[15] In periodontitis sites where IL-4 levels were found to be too low, there was persistent accumulation of activated macrophages, leading to increased destruction of the periodontium.

An early and reliable detection of any adverse peri-miniscrew tissue reaction is essential for patients being treated with miniscrew. Despite investigative efforts to identify the levels of several cytokines in the PMICF, the efficacy of these parameters to predict or to contribute to the diagnosis of peri-implantitis is still undetermined. Hence, the investigators are interested to evaluate the levels of interleukin 8 and interleukin 4 around miniscrew implants during orthodontic tooth movement and to draw clinical inferences from the same.

## MATERIALS AND METHODS

Source of data

The study subjects were patients seeking fixed orthodontic treatment from the Department of Orthodontics and Dentofacial Orthopaedics, Coorg Institute of Dental Sciences, Virajpet, Karnataka. The nature and purpose of the study and the treatment protocol was explained to the subjects included in the study and a written consent from all patients / parents of patients under the age 18 was obtained before commencing the study.

The study sample comprised of 22 miniscrew implants (12 in maxilla and 10 in mandible) from 7 patients who were undergoing orthodontic treatment with miniscrew implants. The selected subjects were in retraction stage using mini-implants [Figure 1]. The miniscrew implants were titanium implants (B K Surgicals) of the size 1.5mm diameter, 8mm long [Figure 2] and was placed by the same orthodontist in all the cases. A force of 150g (measured using dontrix gauge) was applied on each miniscrew implant for retraction.

Inclusion criteria

Patients seeking fixed orthodontic treatment with miniscrew implants

- In the age group 16 to 25.
- With periodontal probing depth upto 3mm.
- With no radiographic evidence of periodontal bone loss after a full–mouth radiographic peri-apical examination.

Exclusion criteria

Patients seeking fixed orthodontic treatment with miniscrew implants

- With periodontal problems.
- With gingival inflammation.
- With any systemic diseases.
- Who have taken anti-inflammatory and antibiotic medications during the past 3 months.

Armamentarium:

PMICF collection-

1. Graduated capillary pipettes
2. Eppendorf tubes

Elisa analysis-

1. ELISA reader
2. Microplate
3. Micropipette
4. Disposable micropipette tips
5. ELISA kit (Elabscience® human interleukin 8 and interleukin 4)

Sample Collection:

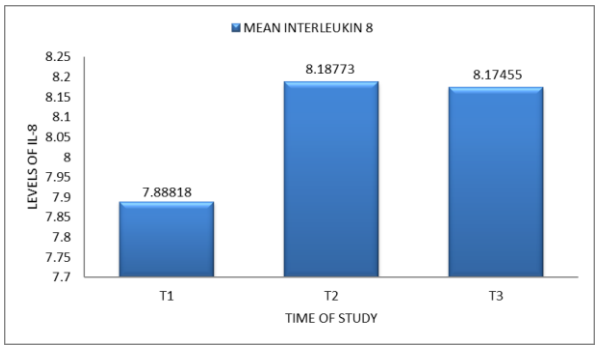
Before commencement of study, the patients were instructed oral hygiene methods and also were systematically checked for periodontal problems. An oral prophylaxis was performed 1 week prior to study. Peri-miniscrew implant crevicular fluid (PMICF) was collected by isolating the area with cotton rolls, drying the adjacent marginal gingiva and teeth with air and using graduated micro capillary tubes from the mesial aspect of the miniscrew implants [Figure 3].[9] The samples were collected over a month time period according to the following schedule: T1, T2 and T3 at one hour, 7$^{th}$ day and 21$^{st}$ day after force application on miniscrew implants respectively. The samples were stored in sterile eppendrof tubes at -20$^{0}$ Celsius until the start of the experiment in Coorg Institute of Dental Sciences. Quantitative analysis of IL-8 and IL-4 in the PMICF samples was assessed using a commercially available ELISA test (Elabscience® human interleukin 8 and interleukin 4). A calibration curve was plotted by regression analysis and the optical density of each sample was used to estimate the concentration of IL-8 and IL-4. This was corrected for the original volume of PMICF and the result was recorded as the concentration of interleukin-8/4 in the samples, expressed in pg/ml.

Statistical Analysis

The data were collected, coded and fed in the SPSS (IBM version 23) and were analyzed using descriptive and inferential statistics. The inferential statistics included paired t test.

## RESULTS

Interleukin 8 level increased from 1 hour to 7$^{th}$ day which is statistically highly significant with p value < 0.01. There is a slight decrease in interleukin 8 levels from 7$^{th}$ day to 21$^{st}$ day which is statistically not significant with p value > 0.050. The difference between 1 hour and 21$^{st}$ day is significant with p value < 0.05. [Graph 1, Table1]

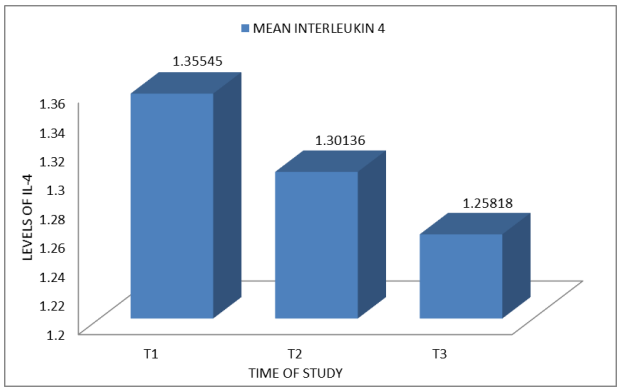



Graph 1: Comparison of mean Interleukin 8 levels at T1, T2, and T3.

Table 1: Comparison of Interleukin 8 levels at different intervals among study subjects around miniscrew implants using paired t test.

| | | Mean | Standard Deviation | t | Sig. |
|---|---|---|---|---|---|
| Interleukin 8 | T1 | 7.88818 | .841505 | -3.630 | **0.002**(H.S)** |
| | T2 | 8.18773 | .930099 | | |
| | T1 | 7.88818 | .841505 | -2.508 | **0.020 *(S)** |
| | T3 | 8.17455 | .992806 | | |
| | T2 | 8.18773 | .930099 | 0.169 | 0.867 (N.S) |
| | T3 | 8.17455 | .992806 | | |

**p<0.01 *p<0.05




Graph 2: Comparison of mean Interleukin 4 levels at T1, T2, and T3.

Interleukin 4 level decreased from 1 hour to 7th day which is statistically highly significant with p value < 0.01.There is a decrease in interleukin 4 levels from 7th day to 21st day which is statistically significant with p value < 0.05. The difference between IL-4 levels at 1st hour and 21st day is highly significant with p value < 0.01. [Graph 2, Table 2]

## DISCUSSION

Interleukin 8 level was used to assess the peri-miniscrew implant tissue inflammation because the various activities of IL-8 indicate that this cytokine plays a major role in mediating inflammatory responses.[8] Sfakianakis et al reported the precise localization of IL-8 receptors in periodontitis and noninflamed human gingivae and suggested that IL-8 plays a multifunctional role in the pathogenesis of periodontal disease.[11] In a previous study by Tuncer BB et al, it was demonstrated that orthodontic forces evoked changes in the levels of IL-8.[12] Evidence from review by Kaur A et al supports high IL-8 levels in periodontitis and at peri-implant inflammation sites.[2] Nowzari H et al IL-8 levels is significantly higher in patients with failing implants than in those with healthy implants.[8, 13]

Table 2: Comparison of Interleukin 4 level among study subjects around miniscrew implants using paired t test.

| | | Mean | Standard Deviation | t | Sig. |
|---|---|---|---|---|---|
| Interleukin 4 | T1 | 1.35545 | .239399 | 3.545 | **0.002** (H.S)** |
| | T2 | 1.30136 | .211306 | | |
| | T1 | 1.35545 | .239399 | 6.445 | **0.000*** (H.S)** |
| | T3 | 1.25818 | .250174 | | |
| | T2 | 1.30136 | .211306 | 2.304 | **0.032* (S)** |
| | T3 | 1.25818 | .250174 | | |

***p<0.001 **p<0.01 *p<0.05

Interleukin 4 level was used to assess the peri miniscrew implant tissue inflammation because Interleukin-4 plays an important role in the regulation of the immunoinflammatory response.[14] Hakami et al reported that the anti-inflammatory effect of IL-4 results from its efficient inhibition of the production of proinflammatory cytokines such as tumor necrosis factor-a (TNF-a), IL-1a, IL-1b, IL-6 and IL-8 by monocytes/ macrophages.[15] It has been hypothesized by researchers that localized absence of IL-4 at the site of gingival inflammation plays a fundamental role in the progression of gingivitis to periodontitis.

The IL-8 levels in healthy implants increased from one hour to 7th day. It could be related to the perimucositis due to the injury during insertion of miniscrew implant. These findings agree with the study by Shaama where the author measured intercellular messenger and cytokines that are regulatory for osteoblast and osteoclast function. It

was reported that IL-8 levels in implant crevicular fluid increased from day 1 but decreased by day 10.[16] There was a slight decrease in interleukin 8 levels from 7th day to 21st day. But it was statistically not significant with p value of 0.867. This is also supported by Hamamcı N et al who found that IL-8 levels increased significantly in both the miniscrew and the canine groups at 24 and 48 hours. No statistically significant difference in the levels of IL-8 was noted on 7th and 21st day from baseline in their study.[8] The study finding is also supported by a study which concluded that levelling and distalization of the teeth evoke increases in IL-8 levels in the periodontal tissues that can be detected in gingival crevicular fluid and distalization forces increased IL-8 levels at the 7th day and decreased on the 21st day.[17]

The IL-8 level decrease from 7th to 21st day but it was not statistically significant (p value>0.05). It indicates that the progress of inflammation is slightly reduced or regressed. This can be attributed to fact that there is no progression towards peri-implantitis. This could be stated in accordance with an in vitro study by Bordin et al. The authors evaluated the fibroblasts of patients with peri-implantitis, patients with periodontitis, and healthy subjects. They found that the fibroblasts of the patients with peri-implantitis and periodontitis synthesized more IL-8 and thus presented a more accentuated proinflammatory profile.[18]

It was concluded that in healthy implants the IL-4 levels decreases from one hour to 7th day. From this it can be inferred that there is some amount of perimucositis around the implant due to the tissue injury during insertion of the miniscrew implants. In the present study, the decrease in IL-4 from 7th to 21st day could be attributed to the persistence of inflammation through 21st day. The study findings is consistent with a study conducted by Pradeep et al which concluded that the mean concentration of IL-4 decreased from periodontal health to disease. They suggested that type 2 helper T cell cytokine, as represented by IL-4 was associated with the remission or improvement of periodontal disease.[14]

The findings of the present study is congruent with the findings of another study which found that in periodontally diseased subjects the total amounts of IL-8 were significantly elevated as compared to healthy subjects, whereas IL-4 showed an inverse relationship to periodontal status and higher amounts were found in the healthy group.[19] This study was also supported by the previous studies by Shapira et al and Kabashima et al who reported lack of IL-4 in gingival crevicular fluid from severe inflammatory sites.[20, 21]

## CONCLUSION AND SUMMARY

The present findings demonstrated that the levels of IL-8 and IL-4 could be considered in detecting the stability of miniscrew implants. A longitudinal study can be conducted to assess the levels of IL-8 and IL-4 with clinical correlation of implants. Further studies are required to elaborate on the levels of interleukins in different condition such as in health, in progression to peri-implantitis and during peri-implantitis.

The study was done around 22 healthy miniscrew implants from 7 patients (6 females and 1 male) within an age range between 16-25 years. The samples were collected over a month time period after force application on mini-implants according to the following schedule: T1, T2 and T3 at 1 hour, 7th day and 21st day respectively, after force application on miniscrew implant. The samples were stored in sterile eppendorf tubes at -20$^0$ Celsius until the start of the experiment. The GCF samples were put through Elisa test (Elabscience® human interleukin 8 and interleukin 4) for determining the concentration of IL-8 and IL-4. The results were analysed using descriptive and inferential statistics.

It was observed that in healthy miniscrew implants the Interleukin 8 levels increased from one hour to 7th day. It could be related to the perimucositis due to the injury during insertion of miniscrew implant. The IL-8 level slightly decreased or remained the same from 7th to 21st day indicating that the progress of inflammation is reduced or regressed. This can attribute to fact that there is no progression towards peri-implantitis. The IL-4 levels in healthy implants decreased from one hour to 7th day. From this it can be inferred that there is some amount of perimucositis around the implant due to the tissue injury during insertion of the miniscrew implants. The IL-4 levels the decreased from 7th to 21st day. This could be attributed to the persistence of inflammation through 21st day.

These findings demonstrated that the levels of Interleukin 8 and Interleukin 4 in PMICF around could be considered in detecting the stability of miniscrew implants.

## CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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