OSTEOPONTIN AND PERIOSTIN LEVELS IN PERI-MINISCREW IMPLANT CREVICAL FLUID DURING CLASS III TREATMENT: AN IN-VIVO STUDY

Sara Elkabbany a, Olfat Shaker b, Nora Abdelgawad c

ABSTRACT

Introduction: Anchorage control is important for the success of orthodontic treatment. The stability of miniscrew implants (MIs) is determined by the clinical, biomechanical and biochemical assessments.

Purpose: The aim of this study was to evaluate the levels of Osteopontin (OPN) and Periostin (PSN) in peri-miniscrew implant crevicular fluid (PMICF) at different time intervals.

Subjects and methods: Eight skeletal Class III patients with maxillary deficiency were selected. Sixteen MIs (Hubit co, Korea) of 1.6 mm diameter and 10 mm length were placed bilaterally between the maxillary second premolars and first molars. Additional sixteen MIs of 1.4 mm diameter and 8 mm length were inserted between mandibular canines and first premolars. A fixed posterior bite plate was used to facilitate bite jumping. 250-300g force per side was immediately delivered by intermaxillary closed coil springs (Ortho Technology, TAD coil spring, USA). PMICF samples were obtained before loading (T1); on day one (T2), two (T3), seven (T4) and on day 30 (T5) after force application. Enzyme-linked immunosorbent assay (ELISA) kits were used to determine OPN and PSN levels.

Results: The percentage change in levels of OPN and PSN broadly showed a decrease upon loading of MIs. However, at the end of observation period, there was no statistically significant difference between T1 and T5.

Conclusions: The OPN and PSN levels varied around MIs as a result of force application and may be used as biomarkers for assessing implant stability throughout loading periods. Immediate loading of MIs with intermaxillary closed coil springs for treatment of skeletal Class III patient did not impair implant stability.

Key words: Miniscrew, OPN; PSN, PMICF, ELISA, Class III.

INTRODUCTION

MIs are increasingly used as temporary anchorage devices. Their ease of placement and removal, ability of immediate or early loading, and relatively low cost, expanding their usage in orthodontics.

In recent years, the use of skeletal anchorage for the orthopedic treatment of maxillary retrognathia has increased to enhance maxillary protraction without the dentoalveolar and skeletal side effects of tooth-borne devices.1 The philosophy of skeletal anchorage is that if the reactive forces can be absorbed by skeletal structures and hence the tooth movement can be limited to the desired therapeutic movements.2

---

a. Lecturer at Orthodontic Department, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt.
b. Professor at Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Egypt.
c. Lecturer at Oral medicine, Diagnosis, Periodontology and Radiology Department, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt.
The factors affecting the stability of MI can be grouped into a host, MIs and technique-related factors. The success rate of MIs is largely dependent on such factors governing primary and secondary stability. The primary stability relates to the mechanical holding of MI in the bone, while the secondary stability relates to biological retention.  

PMICF is an inflammatory exudate that surrounds the MIs crevice with a composition similar to the gingival crevicular fluid (GCF), comprising of inflammatory biomarkers. These are interleukins (ILs) (IL-1β, IL-2, IL-6, IL-8), growth factors and other proteins such as tumor necrosis factor (TNF)-α, receptor activator of nuclear factor kappaB ligand (RANKL), chondroitin sulphate (CS) and osteoprotegerin (OPG).  

In a previous study, a rise in IL-1β levels was seen immediately after MI placement and 24 h after loading. On the other hand, the levels of RANKL and OPG were measured in PMICF. At 24 h, a variation in OPG levels were observed while RANKL level was higher in the loaded group than unloaded group. Moreover, it has been found that applying orthodontic force on healthy MI did not significantly affect CS levels. 

MI insertion in the bone stimulates an immediate host response in the form of clot formation where cellular migration occurs including osteoprogenitor cells, angiogenesis and protein rush including osteopontin (OPN), bone sialoproteins and glycosaminoglycans. At a microscopic level, a hypothesis of micro-crack propagation in the bone upon MI insertion has been proposed. Then the repair is believed to occur by a micro-callus formation triggered by calcium phosphate leading to the creation of mineralized bone.

Osteopontin (OPN) is a highly phosphorylated and glycosylated sialoprotein which is expressed by several cell types including osteoblasts, osteocytes, and odontoblasts. It belongs to the family of non-collagenous proteins known as SIBLING (small integrin-binding ligand, N-linked glycoprotein).

In humans, OPN is encoded by Spp1 gene located on the long arm of chromosome 4 region 22 (4q1322.1). It is a prominent component of mineralized extracellular matrices (ECM) of bones and teeth. It has been found to be involved in bone remodeling, biomineralization, wound healing, apoptosis, and tumor metastasis. Even though OPN was considered as mineralization inhibitor, it has been shown that it can serve as an agent for intra-fibrillar mineralization in collagen, thus indicating the multifunctional role of OPN.

On the other hand, Periostin (PSN) is a matricellular protein which is produced by fibroblasts as a component of the ECM. It facilitates cell–matrix interactions to promote cell survival, angiogenesis, invasion, and metastasis, regulates collagen I fibrillogenesis and interacts with other ECM proteins.

PSN is specifically expressed in periosteum that functions in bone modeling, remodeling and bone repair. Its expression increased in the periodontal ligament (PDL) during initial stages of orthodontic tooth movement. It is essential for homeostasis and remodeling of the periodontium following mechanical stress.

These biomarkers released in PMICF play a very significant role to ensure the secondary stability of MIs. Two recent reviews have attempted to generate evidence on biomarkers.
in PMICF in orthodontic patients. It has been found a total of six studies including IL1β, IL-2, IL-6, IL-8, TNF-α, CS and RANKL/OPG ratio.\textsuperscript{7,19}

Moreover, a recent study highlighted the role of transforming growth factor-beta one (TGF-β1) in bone metabolism around MIs reflecting the state of inflammation from one hour post-implantation.\textsuperscript{20}

Although these few previous studies which evaluated the proinflammatory cytokines in MICF, less is known about the biomolecular level that affects implant stability. Hence, according to our knowledge, this study was the first to evaluate the levels of Osteopontin and Periostin in PMICF before and after loading of MIs with intermaxillary closed coil springs used for orthopedic treatment of skeletal class III patients.

**MATERIAL AND METHODS**

The present study was approved by Research Ethics Committee of the Faculty of Dental Medicine for girls, Al-Azhar University.

A sample size of 32 miniscrew implants was estimated using the power calculation analysis at $\alpha = 0.05$ significance level and $\beta = 0.20$ effect size with 80% being the power of the study using G*Power software (version 3.1.9.2, Franz Faul, Kiel University, Germany).

Accordingly, eight skeletal class III patients (4 males and 4 females), of 12-15 years old with mean age of 13.6 ± 1.68, who required maxillary protraction were selected from the outpatient clinic of Orthodontic Department, Faculty of Dental Medicine for Girls, Al-Azhar University. Written informed consents were obtained from the patients’ guardians.

All patients were in good general health and healthy periodontium with generalized probing depths not exceeding 3 mm and no radiographic evidence of periodontal bone loss.

Sixteen Self-drilling titanium MIs (Hubit co, Korea) of 1.6 mm diameter and 10 mm length were placed bilaterally into the interradicular bone between the maxillary second premolars and first molars in the attached gingiva below the mucogingival junction.

To reduce root contact, the implants were placed in an oblique direction buccolingually, 30° to 40° to the long axis of the teeth in the maxillary posterior area. Additional sixteen MIs of 1.4 mm diameter and 8 mm length were used in lower arch between canines and first premolars in the attached gingiva. Accordingly, each patient had four miniscrews, one in each quadrant.

A fixed posterior bite plate was placed using chemical curing glass ionomer [Kromoglass, LASCOD, Italy] to eliminate occlusal interferences and facilitate bite jumping.

A loading force between 250 and 300g per side was immediately applied by nickel titanium closed coil springs (Ortho Technology, TAD coil spring, 9mm, USA) using force guage [DTC, orthodontic guage force meter, China]. Fig.1
Biochemical evaluation:

PMICF samples were obtained-one hour after Mls insertion at T1 (day 0, before loading) then on day one(T2), day two(T3), day seven(T4) and on day 30 (T5) after force application.

The samples were collected in the early hours of the day to prevent any variations affecting the crevicular fluid volume. Isolation of the Mls site was performed with a cheek retractor, cotton rolls followed by removal of plaque and a gentle air spray around Mls then paper points #35 (Protaper, Dentsply, USA) were inserted into the crevice until mild resistance was felt.

The paper points were left for 30 seconds then transferred to 1.5-ml Eppendorf tubes. Extreme care was taken not to cause any slightest harm or injury during the sample collection. The saliva or blood contaminated samples were excluded. These samples were stored at -20°C and then transferred to -80°C until analysis using enzyme linked immunosorbent assay (ELISA) for assessment of OPN and PSN levels in PMICF.

The collected samples were assayed with (ELISA) kits (Human Osteopontin ELISA kit, E1525Hu) and (Human Periostin ELISA Kit, E3226Hu, Bioassay England/China)

Statistical analysis

Statistical analysis was performed with IBM® SPSS® Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp). Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. The data showed parametric (normal) distribution. Repeated measure ANOVA test was used to compare between more than two groups in related samples. Paired sample t-test was used to compare between two groups in related samples. The significance level was set at P <0.05.

Results

As regard Osteopontin; there was a statistically significant difference among different time intervals (T1 to T5) where (p<0.001). A statistically significant difference was found between T1 and each of T2, T3 and T4 where (p<0.001), (p<0.001) and (p=0.001) and also between T5 and each of T2, T3 and T4 where (p<0.001), (p<0.001) and (p=0.014).

No statistically significant difference was found between any other groups.

Regarding Periostin; there was a statistically significant difference among different time intervals (T1 to T5) where (p=0.034). A statistically significant difference was found between T1 and (T4) where
(p=0.011) and also between T5 and each of T2, T3 and T4 where (p=0.041), (p=0.023) and (p=0.048). No statistically significant difference was found between any other groups. (Table 1, Fig. 2, 3).

**Table (1): Descriptive statistics of Osteopontin and Periostin levels (Pg/ml) and results of repeated measures ANOVA test for comparison at different time periods for each biomarker**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Osteopontin</th>
<th>Periostin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>T1</td>
<td>1446.75 (^a)</td>
<td>223.49</td>
</tr>
<tr>
<td>T2</td>
<td>1040.50 (^b)</td>
<td>95.73</td>
</tr>
<tr>
<td>T3</td>
<td>999.38 (^b)</td>
<td>39.94</td>
</tr>
<tr>
<td>T4</td>
<td>1130.38 (^b)</td>
<td>107.90</td>
</tr>
<tr>
<td>T5</td>
<td>1365.75 (^a)</td>
<td>154.29</td>
</tr>
</tbody>
</table>

\(p\)-value: \(<0.001^*\) \hspace{1cm} 0.034^*  
(SD) standard deviation, \(^*\): Significant at \((p<0.05)\), Means with different superscripts in the same column indicate statistically significant change by time.

**Figure (2): Line chart representing mean values for Osteopontin and Periostin levels in different time periods**
Figure (3): Bar charts representing mean and standard deviation values for Osteopontin(A) and Periostin(B) levels in different time periods.

**Percentage changes (%) in Osteopontin and Periostin levels (Table2, Fig.4,5)**

As regard Osteopontin; there was a statistically significant change at different time periods where (p<0.001). A statistically significant difference was found between (T1-T2) and each of (T2-T3), (T3-T4) and (T4-T5) where (p<0.001), (p<0.001) and (p=0.001). Also, a statistically significant difference was found between (T2-T3) and (T4-T5) where (p=0.025). No statistically significant difference was found between any other groups.

Regarding Periostin; there was a statistically significant change at different time periods where (p=0.007). A statistically significant difference was found between (T4-T5) and each of (T1-T2), (T2-T3) and (T3-T4) where (p=0.005), (p=0.025) and (p=0.030). No statistically significant difference was found between any other groups.
Table (2): Descriptive statistics and results of comparison between percentage changes in Osteopontin and Periostin levels (%)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Osteopontin</th>
<th>Periostin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>T1-T2</td>
<td>34.56 a</td>
<td>6.07</td>
</tr>
<tr>
<td>T2-T3</td>
<td>9.53 c</td>
<td>2.32</td>
</tr>
<tr>
<td>T3-T4</td>
<td>12.76 bc</td>
<td>5.96</td>
</tr>
<tr>
<td>T4-T5</td>
<td>19.56 b</td>
<td>4.34</td>
</tr>
</tbody>
</table>

Means with different small letters in the same column indicate significant difference. *: significant (p<0.05)

Figure (4): Bar chart representing percentage changes (%) in Osteopontin(first) and Periostin(second) levels.

Figure (5): Line chart representing percentage changes (%) in Osteopontin(below) and Periostin(above) levels.
Discussion

MI s are increasingly used as an orthodontic anchorage. Mechanical stimulation of MIs can initiate and promote bone remodeling. Only a few studies have evaluated the proinflammatory cytokines in MICF, monitoring the health status of MIs. (8-10), (22-24)

OPN is considered to play an important role in bone formation and resorption while PSN is essential for the integrity and function of the periodontal ligament in response to mechanical stress.

Therefore, the aim of this study was to determine the OPN and PSN levels around MIs used for direct anchorage to support intermaxillary closed coil springs for treatment of skeletal Class III patient.

In this study, MIs were placed in the attached gingiva to prevent tissue inflammation. Insertion of the MIs below the mucogingival junction allowed a relatively self-cleaning area by physiologic movements. As a result, all MIs survived until the end of the observation period.

In contrast to dental implants, MIs can be loaded immediately with orthodontic forces without complications. Only a few studies, mostly on animals, have dealt with the tissue reactions to immediate loading of MIs. 3,9,(25-27)

In the present study, 250 to 300g force magnitude (per side) was directly delivered by intermaxillary closed coil springs to induce maxillary protraction.

Direct loading was used in most of studies 3,9, (25-27) except in a study 4 assessing IL-1β levels which used indirect loading. Levels of IL-2, IL-6 and IL-8, TNF-α, OC, RANKL and OPG were analysed by applying a force of 150 g 9, (22-24), while 50 g of force was applied to study the levels of CS. 10 Application of different force magnitudes were used to evaluate IL-1β levels; 200g force was used in one study 9 and 120 g force applied in another study 28

Paper strips, paper points, periopaper and micro-capillary pipettes are non-invasive methods of PMICF collection to detect changes during bone remodeling. 8

The MICF samples were collected using paper points in 32 MIs sites before and during immediate loading at different time intervals. i.e., upon MIs insertion, day 1, day 2, day7 and day 30 post loading.

As regards means of OPN; there was a statistically significant change in OPN levels at different time periods (P-value <0.001) Table 1. There was a statistically significant decrease in OPN level from base line (1446.75) to day one (1040.50) followed by non-statistically significant change from day1,2(999.38), to 7 days. From day7 (1130.38) to 30 days (1365.75); there was a statistically significant increase in OPN level to reach base line level.

The decrease in OPN level from base line (T1) to day two(T3) could be attributed to inflammation and bone resorption in peri-implant tissues upon MIs insertion. Moreover, micro-crack propagation in the bone has been proposed due to the difference in elasticity between the bone and MIs structure. 7

The increase in the mean of OPN level from day 7 (T4) till recovery at (T5), where baseline was reached in 30 days, could be attributed to the bone remodeling in the form of increased osteoblastic activity and bone deposition around bone implant interface. The repair of these micro-cracks is believed to occur by a micro-callus formation triggered by calcium phosphate leading to the creation of mineralized bone. 7
As regards means of PSN; there was a statistically significant change in PSN levels at different time periods (p=0.034) Table 1. There was a non-statistically significant decrease in PSN level from base line (119.59), day one (84.75), day 2 (53.73) to day 7 (46.64).

From day 7 to 30 days (122.74); there was a statistically significant increase in mean PSN level to exceed base line level. This change could be attributed to increased osteoblastic activity and bone deposition around bone implant interface.

The percentage change in levels of OPN and PSN broadly showed a decrease upon loading of MIs initially due to the trauma of insertion and later upon application of orthodontic forces. This indicating increased osteoclastic resorption. OPN in PMICF depicted 34.56% decrease in level after one day of MI loading and 9.53% decrease after two days. Then OPN showed 12.76% increase after seven days and 19.56% increase after 30 days of loading.

On the other hand, the mean percentage change in level of PSN in PMICF showed a 30.91% decrease after one day of loading and 26.20% decrease after two days then 29.23% increase after seven days and 44.63% increase after 30 days of loading.

Our findings, regarding the decrease in the levels of OPN and PSN upon loading of MIs till two days were supported by the reported initial increases in inflammatory mediators and RANKL in PMICF. In a previous study, IL-1β levels peak in PMICF was seen immediately at MI placement and 24 h after loading, thereby indicating its important role in inflammation. The levels of IL-1β then gradually decrease at 21 days to reach baseline in 300 days.

Moreover, the levels of RANKL and OPG were measured in PMICF. At 24 h, a variation in OPG levels were observed while RANKL level was higher in the loaded group than in unloaded group. This change could be due to inflammation and bone destruction in peri-implant tissues. Changes in the total amount of RANKL can be attributed to displacement toward the direction of force under orthodontic loading.

It has been reported that MIs were subjected to displacement under orthodontic loading which was correlated to the length of the loading period, although the implants remained stable without detectable mobility or loosening. Also, in a 3-dimensional study, it was concluded that movement of miniscrew implants is expected during orthodontic loading. Normal bone turnover and stable bone mass depend on the balance between OPG and RANKL.

At the end of observation period, there was no statistically significant difference between T1 and T5 regarding the levels of OPN and PSN. These results revealed that levels of both biomarkers recovered after 30 days of force application which signifies increased bone deposition and MIs stability. This may be due to the synergistic or antagonistic action of various cytokines leading to fall in levels and cessation of inflammation and restoration of pdl architecture. The current findings of MIs stability were more or less supported by a previous study which reported that CS did not show a significant variation in placement and loading of MIs.

This study was in agreement with previous studies which indicated that orthodontic static force was not detrimental to MIs stability. This implies insignificant bony resorption around MIs when used as a direct anchorage,
and hence, it supports TADs as absolute anchorage devices.

This study was in agreement with a previous study\(^9\) where the OPG and RANKL levels varied as a result of force application. However, the total amount of OPG remained unchanged and the ratio of OPG and RANKL was stable around loaded MIs.

Similar results have also been observed in the level of OC in PMICF samples where the 150 g of static force applied to MIs did not affect their stability.\(^{24}\)

The results of this study were also in consistent with a recent study\(^{35}\) which evaluated the Interleukin-4 (IL-4) and 2bone turnover markers; bone-specific alkaline phosphatase (BALP) and C-telopeptide of type I collagen (CTX-I) levels in PMCF when using 75 and 150 g of distalization force. BALP level remained unchanged while the CTX-I level on day 7 was higher than the before loading level for both force groups. IL-4 level which is anti-inflammatory cytokine did not significantly change during the study period and between the force groups.

In the current study, all miniscrews which immediately loaded with 250 to 300 g survived and their stability was 100%. The same success rate has been found for previous studies; one used 150g force and one \(^{35}\)used miniscrews loaded with 75 and 150 g of distalization force while another study\(^{36}\) used 100 and 200 g force.

It is possible that the magnitude of forces used in these studies were within the optimal force ranges so the stability of MIs was not affected. However, other factors rather than force magnitude could be responsible for the variation in the success rates observed in other studies.\(^{35}\)

It has been observed that MIs partially osseointegrate.\(^{37}\) This was more or less in consistent to our finding regarding the mean PSN level after 30 days (122.74) which exceeded the value of the initial period (119.59). However, this increase was statistically insignificant.

Finally, the current study has showed that orthopedic force might have a minimal influence on bone remodeling and MIs stability.

**Conclusions**

- OPN and PSN can be detected in PMICF samples during the loaded periods and may be used as biomarkers for assessing implant stability.
- The levels of OPN and PSN were observed higher upon MIs insertion and after 30 days of loading.
- Immediate loading of MIs with 250 to 300g forces did not impair implant stability.
- The recovery of both biomarkers could reflect that the used MIs were clinically healthy and stable.

**Recommendations**

Further studies with longer observation periods, different force magnitudes and various bone biomarkers should be performed to understand the biological factors of implant stability.

In future, a microdevice to evaluate the OPN and PSN level can be helpful for detection of the potential MIs stability.

**References**


