Direct vs Indirect Loading: Comparison of Primary Stability of Orthodontic Miniscrew Implants during Orthodontic Tooth Movement – A Biochemical Assay

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Abstract

The aim of the study was to investigate Alkaline phosphatase (ALP) activity in peri implant crevicular fluid (PMICF) in direct and indirect anchorage side during orthodontic tooth movement in humans. Material and Method: A split mouth technique of ten patients requiring all first premolar extractions were selected and treated with absolute anchorage. Enamel-retraction was done using 150 g sentalloy springs at both direct and indirect anchorage side. Maxillary 1st quadrant side acted as direct anchorage site, while second quadrant acted as indirect anchorage site. Peri implant crevicular fluid was collected from mesial crevices of mini implant before initiation of retraction (baseline), and after initiation of retraction on 1st, 7th, 14th and 21st day and the Alkaline phosphatase activity was estimated using ELISA method. Results: There was no significant difference in means value of Alkaline Phosphatase of two groups at any interval. But the mean difference of Alkaline Phosphatase for all intervals between two group was statistically significant. Conclusions: The overall means of Alkaline Phosphatase of direct method was significantly (p < 0.05) higher than indirect anchorage group. Keywords: PMICF, ALP, Mini implants, Direct anchorage, indirect anchorage, Stability, ELISA.

INTRODUCTION

Over the year’s patient compliance remain a questionable factor in clinical orthodontics. Orthodontics found new extended boundaries and envelope with the introduction of mini-screw implants as temporary anchorage devices. Papadopolus [1] recognized the ability of the temporary skeletal anchorage devices to sustain anchorage loads during orthodontic treatment.

Biomechanics of direct and indirect loading of implants came in to clinical orthodontics with the rationale to conserve anchorage of the molars while increasing stability of mini-implants. Stability of implant is major concern for critical anchorage. Migliorati [2] reported that success rate of the mini-screw implants ranged from 71.4% to 100%. Initially, peri-mucositis a reversible inflammation of the soft tissues surrounding the mini-screw implants occurs, which if left untreated may progress to peri-implantitis. Peri-implantitis accounts for about 30% of mini-screw implant failures. Temporary Anchorage Device placement results in local inflammation due to tissue insult, this inflammatory response is always associated with release of biomarkers in to oral fluids as stated by Krishnan [3] Molecular biology has paved the way to study Blood/Saliva/Gingival Crevicular Fluid and now Peri-Mini Screw Crevicular Fluid.

The concentration of these biologic indicators varied in direct and indirect loading according to Kaur [4] but the study of Holberg [5] reported that taking indirect anchorage is better to minimize the risk of losing mini-screw implants and assessed more stability in indirect loading. Peri-mini screw crevicular fluid (PMCF) is an osmotically mediated inflammatory exudate originating from the vessels of the gingival plexus. Analysis of PMCF offers a non-invasive means of studying host response in and around the device and may provide an indication of patients at risk of losing the micro implant. Alkaline Phosphatase (ALP) plays an important role in bone metabolism. It is a membrane-bound glycoprotein produced by many cells, such as poly-morphonuclear leukocytes, osteoblasts,
macrophages, and fibroblasts within the area of periodontium and gingival crevice. Rawi [6] stated ALP is a very important enzyme as it is a part of normal turnover of periodontal ligament, root cementum and maintenance of these tissues, and bone homeostasis, it is commonly associated with bone metabolism with osteoblasts showing high alkaline phosphatase.

ALP has been studied over the years in orthodontic tooth movement. No study was found in literature wherein ALP was studied in PMICF. This clinical research was designed as a split mouth study to evaluate if differences existed in ALP biomarker levels in PMICF with direct and indirect loaded mini-screw implants during the 3 weeks period of en-masse retraction in orthodontic patients.

OBJECTIVES

To study differences in biologic responses over a period of three weeks between direct and indirect loaded mini screw implants during en-mass tooth retraction using Alkaline phosphatase levels in peri mini screw crevicular fluid as an indicator.

MATERIAL AND METHODS

Clinical, prospective study with split mouth design to assess the biochemical assay of anti-inflammatory biomarker ALP in peri implant crevicular fluid of 10 orthodontic patient over a period of three weeks in orthodontic clinic of Divya Jyoti Dental college Delhi India. The study was approved by Institutional Committee Written informed consent was obtained from each participating patient.

INCLUSION CRITERIA AND EXCLUSION CRITERIA

- Adult patient 18-25 years of age with good systemic and oral health.
- Healthy periodontal tissues with generalized probing depths of ≤3 mm.
- No radiographic evidence of periodontal bone loss.
- Absence of any systemic condition that could affect periodontal status and bone metabolism
- Patient on anti-inflammatory therapy were excluded
- Sample containing saliva and blood were excluded.

METHODOLOGY

Thorough oral prophylaxis was done before starting treatment. Patients were instructed not to take any non-steroidal anti-inflammatory drugs during the study period. The first premolar extractions were done at the start of treatment or at least one month before the commencement of en-masse retraction so that the bone remodelling occurring due to the healing socket would not influence our study. The patients were treated with conventional straight wire (022” slots) MBT mechanotherapy. Levelling and alignment was done using 016 NiTi arch wires.

A total of 20 implants were placed bilaterally between maxillary second premolars and first molars in the attached gingiva at the mucogingival junction. Topical anesthesia was given before implant placement. Bracket head mini screw implants of length 8mm and width 1.8mm were placed by self tapping technique at an angle of 45° both sides simultaneously.

Direct Anchorage

Implant placed between second premolar and 1st molar on maxillary right quadrant were used for direct anchorage where in closed coil spring for retraction was placed directly from power arm to the mini implant.

Indirect Anchorage

Implant placed between second premolar and 1st molar on maxillary left quadrant used for indirect anchorage where in mini-implant was stabilized with stainless steel 17 × 25 sectional wire to first molar auxiliary tube to restrict forward movement of 1st molar on same side and retraction coil was placed from retraction arm to the hook of molar band of same side. The retraction of the anteriors as en-masse was initiated on a working base wire of 019 x 025” Stainless En-masse retraction was performed, using miniscREW implants and Nitinol closed coil spring (8 mm) capable of delivering 150 g of constant force.

PMCF (Perimini screw implant crevicular fluid) was collected around the mini implant by using capillary action of micropipette Table-1; Collected Sample were stored in minus 70 degree until the last sample of the study was collected and assayed with enzyme-linked immunosorbent assay (ELISA) kit.

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RESULTS

In direct anchorage group mean value of Alkaline Phosphatase decreased at day 1 Compared with day 0 and then showed steady increase till 21 days. However the mean value at day 21 was more than time interval at day 0. In indirect anchorage group mean value of Alkaline Phosphatase decreased at day 1 in comparison to day 0 and showed steady increase till 21 day. However the mean value at 21 day was less than 0 day On Comparison of mean of Alkaline Phosphatase in Direct anchorage and indirect anchorage group between different time intervals by repeated measure of ANOVA, there was no significant difference (Sphericity assumed) in mean of Alkaline Phosphatase in Direct method between different time intervals, p>0.05. On Multiple Comparison of means of Alkaline Phosphatase in both Direct and indirect anchorage groups between two time intervals by was not significant, p>0.05 Bonferroni test. Table-2, and Independent t test Table-3. There was no significant difference in means Alkaline Phosphatase of two groups at any interval, p>0.05, Table-3. While the mean difference of Alkaline Phosphatase of all intervals between two group is significant .So, over all Mean of Alkaline Phosphates of Indirect anchorage is significantly less than in direct method. The overall means of Alkaline Phosphatase of Direct group is significantly higher than Indirect group, p<0.05 v Bonferroni test Table-4.

Table-2: Comparison of over all of mean of Alkaline Phosphatase of two anchorage groups between two time intervals by Bonferroni test

<table>
<thead>
<tr>
<th>(I) Interval</th>
<th>(J) Interval</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>P value</th>
<th>95% Confidence Interval for Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>T2</td>
<td>-.009</td>
<td>.010</td>
<td>1.000**</td>
<td>-.039 - .022</td>
</tr>
<tr>
<td>T1</td>
<td>T3</td>
<td>-.012</td>
<td>.010</td>
<td>.721**</td>
<td>-.041 - .016</td>
</tr>
<tr>
<td>T2</td>
<td>T3</td>
<td>-.004</td>
<td>.013</td>
<td>1.000**</td>
<td>-.041 - .034</td>
</tr>
</tbody>
</table>

Table-3: Comparison of mean of Alkaline Phosphatase between anchorage at different time intervals by Independent t test

<table>
<thead>
<tr>
<th></th>
<th>t-test for Equality of Means</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>Mean Difference</td>
<td>Std. Difference</td>
<td>Error</td>
<td>95% Confidence Interval of the Difference</td>
</tr>
<tr>
<td></td>
<td>(2-tailed)</td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>T1</td>
<td>-.009</td>
<td>.013</td>
<td>.0098</td>
<td>-.01328</td>
</tr>
<tr>
<td>T2</td>
<td>-.012</td>
<td>.015</td>
<td>.02150</td>
<td>.01559</td>
</tr>
<tr>
<td>T3</td>
<td>-.004</td>
<td>.013</td>
<td>.01930</td>
<td>.01526</td>
</tr>
</tbody>
</table>

Table-4: Comparison of over all of mean of Alkaline Phosphatase of between methods of different time intervals by Bonferroni test

<table>
<thead>
<tr>
<th>(I) Group</th>
<th>(ID) Group</th>
<th>Mean Difference (1-J)</th>
<th>Std. Error</th>
<th>P value</th>
<th>95% Confidence Interval for Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td>Indirect</td>
<td>.017</td>
<td>.006</td>
<td>.015*</td>
<td>.004 - .030</td>
</tr>
</tbody>
</table>

DISCUSSION

The idea of using implants to improve orthodontic anchorage was first published by Gainsforth and Higley [7] in 1945. Papadopoulos et al., [1] described miniscrew implants are becoming increasingly popular in orthodontics because they provide absolute and skeletal anchorage for orthodontic tooth movements. Other advantages include their low cost and simple surgical placement and removal. convenient size of the miniscrew implant also enables their use in many anatomical regions, including the The small and interdental area TADs work by two biomechanical approach, either direct anchorage or indirect anchorage. Direct anchorage as stated by Razavi [8] describes situations where the teeth desired to be moved are pitted directly against the TADs. The direct anchorage force systems are easy to design, and require no laboratory appliance fabrication. In most surveys miniscrews were loaded directly, but due to the relatively high failure rate (15– 30 %) reported by Migliorati et al., [2] led to the implementation of an indirect anchorage concept. Indirect anchorage on the other hand, refers to the stabilization of certain teeth in the dental arch, and subsequent use of these stabilized anchors to move other teeth in the dental arch.

Alkaline Phosphatase is a membrane-bound glycoprotein produced by many cells, such as poly-morphonuclear leukocytes, osteoblasts, macrophages, and fibroblasts within the area of periodontium and gingival crevice The present study used ALP an anti inflammatory marker in PMCF using immunoassay technique for assessing mini-screw implant stability as it is expressed early during bone synthesis process as reported by Weinreb et al., [9] stated that ALP is an earlier differentiation marker than osteopontin and osteocalcin during the formation of endochondral and membranous bone. Significant interest in Alkaline Phosphatase expression has also come from tissue engineering experiments, where enzyme expression is a
good predictor of neotissue mineralization. That is the reason ALP was chosen as a biomarker to assess implant stability using periimplant crevicular fluid as a sample.

Rawi et al., [6] stated Alkaline phosphatase activity is important for the mineralization of bone and represents a useful biochemical marker of bone formation. Sekhar et al., [10] reported Alkaline phosphatase is commonly associated with bone metabolism with osteoblasts showing high alkaline phosphatase. Acid and alkaline phosphatases are released by injured, damaged, or dead cells into extracellular tissue fluid. Alkaline phosphatase was effectively seen in oral fluids (GCF and saliva) and was determined as effective biomarker to assess alveolar bone remodeling during orthodontic tooth movement and periodontal disease

Peri mini screw crevicular fluid was chosen the sample to detect Alkaline Phosphatase (a bone synthesizing marker because, Enhos et al., [11] stated that the composition of peri-miniscrew implant crevicular fluid (PMCF) is analogous to the gingival crevicular fluid. PMICF is in close proximity contact with the mini screw implant inserted into the tissues and, could reflects much biochemical response in them than GCF and Saliva. Literature has shown various methods of GCF collection as paper strip method by Wahab et al., [12], but Kaur et al., [4] stated capillary method as easy and non invasive method of collection of collection GCF. So the present study used non invasive capillary method for collection of PMCF as a sample for biomarker assessment.

Orthodontic tooth movement is considered an epiphenomenon of the gene expression of the periodontal ligament. A chemical cascade that mediates the transmission of signals from extracellular matrix leading to genetic modulation is interceded by the release of mediators in paracrine environment. These signals are responsible for a change in the cytoskeletal structure, leading to alteration of nuclear protein matrix and eventually gene activation or suppression these events initiate the process of bone remodeling, leading to effective tooth movement as stated by Krishnana [3]. The time interval for sample collection of present study was at day 0, day 1, day 7 and day 21 .This can be explained by the physiologic response in periodontium after the orthodontic force application. In particular, it has been shown that in the early phases of bone remodeling, a resorption activity (3-5 days) is followed by its reversal (5-7 days) and, subsequently, by a late phase of bone deposition (7-14 days) in both tension and pressure sites of the alveolar wall. In the early phase of tooth movement, bone resorption is greater than bone deposition, but in a later phase, resorption and deposition could become synchronous. This might be due to the high acid phosphatase activity that has been observed in the early period of tooth movement; high levels of ALP activity have been described after 7 days, when bone deposition begins.

ALP is considered to be a marker. Of osteoblastic activity because this enzyme is essential for bone deposition it hydrolyzes nonorganic pyrophosphate, which is a potent inhibitor of the mineralization process. Different results have been reported in studies investigating ALP activity in the periodontium of teeth undergoing orthodontic treatment by Perinetti et al., [13] and showed increased activity of ALP activity from 7 to 28 days. Batra et al., [14] reported the rise of ALP in 7 and 21 day of orthodontic retraction we used bracket head mini implants in split mouth design technique, direct on maxillary 1st quadrant and indirect anchorage on 2nd maxillary quadrant, so that a patient himself act as control to assess the biomarker changes in same individual. Huang et al., [15] stated the use of bracket head implants for direct or indirect anchorage as well This study is first ever study in literature where direct and indirect anchorage was taken to assess the bone forming biomarker ALP changes in PMCF. Wahab et al., [12] has done similar study of split mouth and has studied the effect of different orthodontic force during tooth movement.

The present study, studied the biomarker ALP and found the presence of ALP at all-time interval in both direct and indirect methods. This suggests that this biomarker (ALP) can be used to assess the alveolar bone modeling around mini screw implant in direct and indirect anchorage groups. During the Day of implant placement, the presence of ALP activity could be attributed to the inflammatory response caused by the trauma to the soft tissues during implant placement .The mean value on the same Day between two groups was not statistically different. After 1 day of loading, the mean value of ALP was decreased in both the groups. This could be attributed to biological inflammatory response occur due to loading of mini screw implants. Comparing the mean value at Day 0, with mean value of Day 1 in direct anchorage group and indirect anchorage group, the mean value showed slight decrease between two time intervals. On 7th day of loading the mean of ALP was increased in both the groups. This can be explained by biological response at early phases of bone remodeling during tooth movement, resorption activity 3 to 5 Days is followed by its reversal 5 to 7 Days and, subsequently by a late phase of bone deposition 7 to 14 Days in both tension and pressure sites of the alveolar wall. The mean value of direct group is comparatively higher in direct group than indirect group. Comparing the mean value at Day 7 with the mean value at Day 0, showed steady increase in mean value in both direct and indirect anchorage groups between the two time intervals. On Day 21 the mean value of ALP showed steady increase in both the groups this could be attributed to adaptive bone response to the orthodontic forces. The mean value in
direct group is comparatively higher than indirect group. Comparing the mean value at Day 21 with the mean value at Day 0, it showed constant increase in mean value in both direct and indirect anchorage groups between the two time intervals. The mean value of ALP in direct anchorage group at Day 21 was higher than the mean value at Day 0 than indirect group which shows more stability toward direct group than indirect group. There was no statistical significant difference found between two groups but ALP level was comparatively higher in direct anchorage group.

Due to lack of literature evidence on ALP levels in PMICF, the result of current research may be compared with the studies on OTM using ALP biomarker in GCF. The results of this study was supported by the studies done by Perinetti et al., [13] wherein they showed the increase in ALP activity from 1st week to 4th week, Wahab et al., [12] reported the peak level of ALP on 2nd week, Batra P et al., [14] showed steady increase on 7th, 14th and 21st Day, Asma et al., [16] showed slight increase till 8th week and Insof et al., [17] also showed more ALP activity during orthodontic tooth movement between 1 to 3 week of orthodontic tooth movement.

Osteocalcin (OC) another important anti-inflammatory bone forming biomarker studied by Danashetti et al., [18] in PMICF wherein they showed significantly increase in level of OC from Day 0 to Day 7 but statistically non significant increase from Day 7 till 30th day . Similar study by Alfaqeeh et al., [19] found OC in GC F showed statistically significant changes in OC levels on days 7, 14, and 21, and showed peak in activity of the variables occurred on day 14 during orthodontic retraction. It could be concluded that the overall mean of ALP of direct anchorage group was significantly higher than indirect anchorage group, higher the Alkaline Phosphate more is the bone deposition as stated by Asma et al., [16], therefore rise in ALP may depict more stability of mini implants in direct anchorage group. However these findings are in contrast with the study done by Holberg et al., [5] which stated that it is better to choose an indirect anchorage in order to minimize the risk of losing mini-screw implant.

**CONCLUSION**

The present study showed the presence of ALP at all-time interval in both direct and indirect methods. This suggests that this marker can be used to assess the alveolar bone modelling around miniscrew implant in direct and indirect group. Steady increase was found in both groups (direct and indirect) the overall mean of ALP of direct group was significantly higher than indirect group. Rise in ALP may depict more stability of mini-implants in direct group. The study has limitation in terms of sample size, the finding of the study need future corroboration on a large sample size and for long duration while considering the enzyme activity in both direct and indirect anchorage group as well, also to know if difference are elucidated in enzyme activity between the two genders during orthodontic tooth movement.

**REFERENCES**


