Comparative Evaluation of Osteocalcin in Peri-implant Crevicular Fluid and Radiographic Bone Loss in Immediate Loading and Delayed Loading Protocols: A Preliminary Split-Mouth Randomized Controlled Trial

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Purpose: To compare osteocalcin and crestal bone loss in implants placed under an immediate loading (IL) compared to a delayed loading (DL) protocol. Materials and Methods: This preliminary, split-mouth, randomized controlled trial included 14 participants who required replacement of both mandibular first molars opposing a completely dentate maxillary arch. Two implants were placed in each participant. According to the split-mouth randomization method, a temporary crown was used for the IL protocol and a healing abutment was used for the DL protocol in each participant. Definitive crowns were cemented 3 months after implant placement. Osteocalcin levels were determined using ELISA, and crestal bone loss was evaluated using radiographs at 2 weeks, 3 months, and 12 months after implant placement. Results: The mean osteocalcin level was significantly higher with IL than DL at each point (P < .001), with 95% CI of –262.89 to –439.10 (2 weeks); –238.02 to –375.98 (3 months); and –83.24 to –211.61 (12 months). Higher crestal bone loss was observed in IL when compared to DL implants at 2 weeks (P = .458, 95% CI: –0.10 to 0.21). Less crestal bone loss was observed with IL than DL at 3 months (P = .935) and 12 months (P = .42).

Conclusion: Osteocalcin levels increased in both IL and DL implants, but higher levels were observed with IL. Higher crestal bone loss was observed with IL during the initial stages of treatment only. Int J Prosthodont 2022;35:174–180. doi: 10.11607/ijp.7489

A traditional submerged protocol, or delayed loading (DL), for tooth replacement using dental implants has been associated with predictable results and successful outcomes due to lack of micromotion of the implants and the firm union between the bone and the implants. Proponents of this concept discourage premature loading of implants, as the induced micromotion can result in a fibrous union between implant and bone and an eventual compromise of the integration of implants.1,2 Advancements in implant design, materials, and surface treatment have provided options for fast and steady osseointegration.3 Immediate loading (IL) is the placement of a restoration within 48 hours of surgical placement of the implant. The benefits of IL include prompt fulfillment of esthetics and functional requirements of the patients, thus providing psychologic satisfaction. The single most important determinant of IL is the primary stability of implants, which can be achieved by bicortical stabilization under drilling of osteotomy or by use of an osteotome to laterally condense the bone.4,5 Satisfactory clinical outcomes and agreeable survival rates have
been reported with IL when compared to the submerged approach in patients with multiple splinted and single implants.\textsuperscript{6–13}

Experimental animal histologic reports suggest an increase in bone apposition on the implant surface and an increase in bone-to-implant contact when subjected to IL.\textsuperscript{14–16} Such histologic studies cannot be carried out in humans. Commonly used parameters for assessing the status of dental implants are the probing depth of the sulcus, bleeding on probing, suppuration, mobility, and marginal bone loss. However, these parameters are associated with low sensitivity, especially in the early stages of hard and soft tissue changes.\textsuperscript{17} The use of biomarkers in peri-implant crevicular fluid (PICF) to assess the host-tissue response around the implant has been suggested in the literature.\textsuperscript{18,19} Several biomarkers like interleukins, matrix metalloproteinase (MMPs), cathepsin K, vascular endothelial growth factor (VEGF), transforming growth factor $\alpha$ (TGF$\alpha$), osteoprotegerin (OPG), osteocalcin (OCN), osteopontin (OPN), and parathyroid hormone (PTH) can be assessed in PICF for different purposes, such as checking the early inflammatory response, osteogenesis, and osteoclastogenesis around the implant.\textsuperscript{20} OCN is a non-collagenous protein secreted by osteoblasts. It is encoded by the OG1 and OG2 genes, which are also expressed by osteoblasts.\textsuperscript{21} It is known to bind to hydroxyapatite and calcium during matrix mineralization and has an important role in the regulation of metabolism, bone mineralization, and calcium ion homeostasis.\textsuperscript{22}

Clinical outcomes of IL when compared to DL in multiple, splinted fixed prostheses supported by implants have been well documented.\textsuperscript{13,20,23} However, the literature related to the biomarkers associated with IL in a single, unsplinted implant-supported prosthesis in partially edentulous cases is scant. The primary objective of this study was to comparatively evaluate the OCN levels in the PICF of IL and DL implants at 2 weeks, 3 months, and 12 months after implant placement, and the secondary objective was to comparatively evaluate crestal bone loss (CBL) at the same time points. The null hypothesis was that no statistically significant difference would be found in OCN levels between IL implants and DL implants, or in CBL between IL implants and DL implants.

**MATERIALS AND METHODS**

This split-mouth randomized controlled trial was started after obtaining ethical clearance from the Institute Ethics Committee (Reference no.: IECGP-219/28.06.2018) and registration under Clinical Trials Registry-India (CTRI/2019/05/019411). The protocol guideline was undertaken according to the CONSORT (CONsolidated Standards Of Reporting Treatment) statement.\textsuperscript{24} Since previous clinical study results were not available during establishment of the study design, biometric sample size calculation could not be performed. A convenience sample size of 14 was selected for this pilot study. Subjects between the age of 20 and 45 years, irrespective of gender, who required replacement of missing first molars bilaterally in the mandibular arch (duration of edentulousness 6 to 12 months), with a completely dentate maxillary arch, controlled oral hygiene, and adequate bone quality and quantity (bone density of D1 or D2, bone height of 11 to 14 mm, bone width of 6 to 10 mm), as determined by CBCT for implant placement, were selected. The duration of this prospective study was 18 months.

Subjects with parafunctional habits, smoking, irradiation, pregnancy, or with systemic, congenital, or metabolic disorders were excluded from the study. A complete history including clinical and radiographic examinations (orthopantomogram and CBCT) was obtained, and the entire treatment procedure, its benefits, and its complications were explained to obtain informed consent before the procedure was started. Subjects in whom implant insertion torque $\geq$ 35 Ncm was not achieved were also excluded from the study. A computer-generated randomization table (simple randomization) was used with sequentially numbered opaque sealed envelopes for allocation concealment to allocate IL or DL protocols on either side of the oral cavity in each subject. All subjects were treated by a single operator (D.K.) and received a single brand of implants (Touareg S, Adin Dental Implant Systems) after implementation of a common aseptic standard flapless surgical procedure, and all prostheses were fabricated in a single laboratory by using common materials and techniques for standardization.

For the enrolled subjects, oral hygiene instruction was reinforced, and diagnostic models were made for wax-up and surgical guide fabrication. The surgical guide was fabricated by using the vacuum-formed thermoplastic sheet (Biocryl C, Scheu-Dental) for surgical placement of implants using a flapless technique as described by Nanda and Jain.\textsuperscript{25} Two endosseous internal hex dental implants were placed bilaterally in the planned mandibular site using the prefabricated surgical guide in each subject. The insertion torque value for each implant was observed to be $\geq$ 35 Ncm. After surgery, participants were advised to rinse twice a day with 0.12% chlorhexidine mouthwash for 10 days and to consume oral antibiotics (amoxicillin 500 mg) and analgesics (paracetamol 500 mg) thrice a day for 5 days. One of the two implants in each participant (depending on the randomization table) was loaded according to the IL protocol within 48 hours of implant placement. The implant on the opposite site was covered with a mucosal healing abutment, to be followed by rehabilitation after 3 months according to the DL protocol.

In order to fabricate the crown for the IL protocol, an implant-level impression was made in additional silicone.
material (Reprosil, Dentsply Sirona). An indirect transfer coping was connected to the implant, and a single-step double-mix impression technique (Reposil) was used in a custom tray for the impression procedure. A temporary implant abutment (RS Straight Engaging Titanium Abutment, Adin, Dental Implant Systems) was connected to the implant analog. Screw-retained temporary crowns were fabricated using bis-acrylic composite resin material (Protemp 4, 3M Espe). After verification of the proximal contacts and emergence profile, the screw-retained temporary crowns were tightened with 15-Ncm torque, and the access hole was closed using composite resin (Tetric N-Ceram, Ivoclar Vivadent). Passive occlusal contact was developed in maximum intercuspation, and all contacts in the eccentric position were relieved. After 3 months, titanium straight abutments (RS Straight Engaging Titanium Abutment) were connected to the implant, and definitive metal-ceramic restorations were cemented on the abutments using zinc phosphate cement (DeTrey Zinc Cement, Dentsply Sirona) for all participants using standard operating prosthetic procedures. To assess oral hygiene status, the periodontal pocket depth (assessed using a periodontal probe [Williams Colorvue, Hu-Friedy]) and modified Gingival Index around the implants were obtained by a single operator (N.K.) to rule out any interoperator variability.

To evaluate OCN, the PICF was collected from the implant sites at 2 weeks, 3 months, and 12 months after the surgical procedure. Before the collection of samples, the implant sites were isolated with cotton rolls. PICF was collected using PerioPaper strips (Oraflow), as seen in Fig 1. The PerioPaper was inserted into the gingival sulcus until slight resistance was felt and the fluid was adsorbed into the filter paper by capillary action. After this, the adsorbed PerioPaper strip was dipped in 100-µl phosphate-buffered saline in a 500-µL centrifuge tube. The samples were stored at –80°C, and proteins were extracted from all of the strips. A total of 12-µg isolated protein was used per well in the enzyme-linked immunosorbent assay (ELISA). ELISA was carried out for the quantitative estimation of OCN protein in both IL and DL implants. ELISA experiments were performed using standard kits (OCN, RayBiotech), and the assay procedure was followed per the manufacturer instructions.

To evaluate CBL, intraoral periapical (IOPA) radiographs were taken by using radiovisiography. The
long-cone paralleling technique was used for radio-
graphic exposure immediately after implant placement
and at 2 weeks, 3 months, and 12 months after the
surgical procedure. A customized occlusal jig was fabri-
cated on the paralleling device (Rinn XCP-ORA, Dentsply
Sirona) by adapting freshly mixed putty (Aquasil, Dentsp-
ly Sirona) around the bite fork of the device and asking
the participants to bite on the putty. The occlusal jig
was used to ensure repeatable alignment of the po-
sitioning device and the head of the radiograph tube.
In each recorded radiograph, the bone level was mea-
sured around the implant using ImageJ software (version
1.47, National Institutes of Health). Implant length was
used to calibrate and set the scale in the software. This
yielded a pixel-per-millimeter ratio, and the vertical dis-
tance between the shoulder of the implant and the first
bone-to-implant contact was measured based on this
ratio.27 CBL was calculated by subtracting the bone levels
at each follow-up time interval (2 weeks, 3 months, or 12
months) from the bone level immediately after implant
placement for the mesial and distal sides, as seen in Fig
2. The mean bone loss on the mesial and distal sides of
each implant were then determined to obtain the rep-
resentative CBL around each implant. The data collected
were subjected to statistical analysis using statistical
software (Stata version 16.0). Quantitative variables were
summarized as the mean and SD for OCN and CBL. Ap-
proximate normality was tested using Shapiro-Wilk test.
To compare the quantitative values within each group,
repeated-measures ANOVA with Bonferroni post hoc
adjustment was used. To compare the values between
the two groups, paired t test was used (α = .05).

**RESULTS**

A total of 28 implants were placed in 14 subjects. Of the
14 subjects, 9 (64.3%) were men and 5 (35.7%) were
women, with a mean age of 28.7 ± 3.5 years. Oral hy-
giene evaluation via modified Gingival Index and probing
depth in both groups showed no statistically significant
difference in mean values at different time intervals in
either group (P > .05). There were no dropouts over the
12-month follow-up period, and there was no report
of implant or prosthesis failure by any participant. Data
were collected at 2 weeks, 3 months, and 12 months
after implant placement.

Table 1 shows the mean and SD values of the OCN
in PICF around IL and DL implants. An increase in OCN
levels was observed from 2 weeks to 12 weeks in both
protocols. Intergroup comparison showed a significantly
higher OCN level in IL implants compared to DL implants
at all time points (Table 1). Intragroup comparison be-
tween different time points within both groups showed
a statistically significant difference in OCN levels at all
time intervals (Table 2). Table 3 shows the mean and SD
values of the CBL around IL and DL implants. Increasing
CBL was observed over the 12-month period in both
groups. Intergroup comparison showed higher CBL for
IL implants at 2 weeks compared to DL implants, but the
difference was not statistically significant. The amount of

Table 1  Osteocalcin (pg/mL) Levels in Both Groups and Intergroup Comparison at Each Time Point

<table>
<thead>
<tr>
<th></th>
<th>2 wk</th>
<th>3 mo</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate loading</td>
<td>Mean ± SD</td>
<td>591.64 ± 182.81</td>
<td>1,074.86 ± 135.31</td>
</tr>
<tr>
<td>Delayed loading</td>
<td>Mean ± SD</td>
<td>240.64 ± 88.43</td>
<td>767.86 ± 119.29</td>
</tr>
<tr>
<td>Intergroup comparison</td>
<td>P</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>95% CI</td>
<td>–262.89, –439.10</td>
<td>–238.02, –375.98</td>
<td>–83.24, –211.61</td>
</tr>
</tbody>
</table>
CBL for IL compared to DL implants was less at 3 months and 12 months; however, these differences were not statistically significant.

A statistically significant CBL was observed for both IL and DL implants over a period of 12 months. Intragroup comparison between the different time points showed significantly higher CBL at 3 months and 12 months compared to 2 weeks in both IL and DL implants, but the difference between 3 months and 12 months was not statistically significant in either the IL or DL implants (Table 4).

**DISCUSSION**

The first null hypothesis must be rejected because there was a statistically significant difference in OCN levels between the IL and DL implants at 2 weeks, 3 months, and 12 months. The second null hypothesis was accepted, as there was no statistically significant difference found in CBL between IL and DL implants at 2 weeks, 3 months, or 12 months.

Biomarkers in the peri-implant microenvironment have been quantified to develop an early diagnostic technique for peri-implant homeostasis, as well as hard and soft tissue changes. Various bone markers have been evaluated previously for IL and DL implants. The analysis of biomarkers can be considered as an adjunct to the clinical and radiographic diagnostic parameters, which have been associated with low sensitivity and specificity for distinguishing hard and soft tissue disease progress.

The literature suggests that the timing of implant loading influences the bone activity around the implant. OCN is an osteoblast-derived hormone that has been known to bind to the calcium ions in hydroxyapatite and regulate the rate of bone mineral maturation. A change in the intensity of bone activity can thus bring a change in the level of OCN. During the osseointegration of implants, the changing bone physiology is expected to be reflected as a change in the OCN level, as can be seen in the present results. A recent review related to bone turnover markers suggested that OCN level shows an increase with mode and intensity of physical stimulus. A previously reported animal study suggested that OCN is the best biologic marker for implant osseointegration after a 4-week healing period. In another study, the highest gene expression was found for OCN at 7 weeks after implant placement.

The results of this study also show that OCN levels increased significantly with time in both IL and DL implants. Higher OCN levels have been observed in IL and DL implants at 3 months and 12 months, but the difference between 3 months and 12 months was not statistically significant.

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**Table 2** Intragroup Comparisons of Osteocalcin Level (pg/mL) at Different Time Points

<table>
<thead>
<tr>
<th></th>
<th>2 wk–3 mo</th>
<th>2 wk–12 mo</th>
<th>3 mo–12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate loading</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Delayed loading</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

**Table 3** Crestal Bone Loss (mm) in Both Groups and Intergroup Comparison at Each Time Point

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>2 wks</th>
<th>3 mo</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate loading</td>
<td>Mean ± SD</td>
<td>0 ± 0</td>
<td>0.30 ± 0.27</td>
<td>0.87 ± 0.48</td>
</tr>
<tr>
<td>Delayed loading</td>
<td>Mean ± SD</td>
<td>0 ± 0</td>
<td>0.20 ± 0.12</td>
<td>0.88 ± 0.36</td>
</tr>
<tr>
<td>Intergroup comparison</td>
<td>P</td>
<td>.458</td>
<td>.935</td>
<td>.42</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>(–0.10, 0.21)</td>
<td>(–0.30, 0.28)</td>
<td>(–0.02, 0.13)</td>
</tr>
</tbody>
</table>

**Table 4** Intragroup Comparisons of Crestal Bone Loss (mm) at Different Time Points

<table>
<thead>
<tr>
<th></th>
<th>2 wk–3 mo</th>
<th>2 wk–12 mo</th>
<th>3 mo–12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate loading</td>
<td>.005</td>
<td>.003</td>
<td>89</td>
</tr>
<tr>
<td>Delayed loading</td>
<td>.001</td>
<td>.001</td>
<td>79</td>
</tr>
</tbody>
</table>

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implants compared to DL implants. This finding may be due to the greater intensity of bone remodeling as a result of the physiologic bone stimulus due to the functional contact with IL. Similar findings have been reported previously in the literature and are supported by the fact that OCN responds to a mechanical stimulus. The higher level of OCN at 2 weeks and 3 months might be a prognostic indicator of better continuous remodeling. In a study by Prati et al, higher levels of OCN at 7 days and 30 days after implant placement with an IL protocol were seen compared to a DL protocol; however, they observed a higher level of OCN in the DL implants compared to the IL implants after 60 days and 120 days. The contradictory findings of this study may be attributed to the use of a splinted prosthesis. In a multiple-unit splinted prosthesis, the stimulus for bone remodeling is moderated by the splinting of implants as well as the occlusal contacts. In a single-unit, unsplinted prosthesis, the stimulus is largely dependent on the occlusal contacts.

The higher level of OCN in the IL implants compared to the DL implants at 12 months cannot be compared to the existing literature, as previous studies have evaluated changes in OCN level for a maximum follow-up of 3 months. The increase in OCN level in both groups can be attributed to the continuous bone remodeling process. Evidence of continual bone remodeling has been seen in radiographic assessment of interproximal bone levels in many long-term studies.

In the present study, changes in CBL were analyzed with a standardized digital IOPA radiograph using ImageJ software for IL and DL implants at each time interval. The CBL decreased significantly within both IL and DL groups over the 12-month observation period. The resulting decrease in CBL in both protocols was within acceptable limits, as stated by Albrektsson et al. There was a statistically significant difference in CBL at 3 months and 12 months when compared to DL at 2 weeks in both groups, but the difference between 3 months and 12 months was not statistically significant. The findings of the present study are in agreement with other studies, which have reported CBL around an implant in the 12 months after its placement to be in the range of 0.4 to 1.6 mm. A larger amount of CBL in the first 3 months has been attributed to the surgical process and initial healing. The remodeling process during this time is also associated with the formation of large woven bone that is not mineralized and hence lacks the radiopacity to be detected in radiographs. CBL after implant placement has also been attributed to other factors, such as reflection of periosteum, osteotomy, the microgap between implant and abutment, micromovement of the abutment components, bacterial infiltration, the establishment of a biologic width, and stress factors. To curtail these confounding variables in the present study, all implant surgeries and prosthodontic treatments were performed by a single operator using the same standard techniques.

The CBL at 12 months in both groups can be explained by the principle of composite beam analysis. The other important factors that may influence CBL are oral hygiene, including gingival and periodontal health. These factors were verified during participant selection. Additionally, all participants were educated on good oral hygiene practices to maintain satisfactory oral hygiene levels for the entire duration of the study.

Intergroup comparison between IL and DL implants showed a statistically insignificant difference in mean CBL at each time interval. Similar findings have been noted by Güncü et al and Salvi et al. Thus, it can be interpreted that both loading protocols show CBL within the physiologic limit and that the IL protocol has no deleterious effect on crestal bone physiology. Also, since this is a split-mouth study, the biomarkers for both sites were produced in the same individual. This imparts a greater accuracy for the intergroup comparisons than studies where test and control sites were in different individuals.

The limitations of this study were the low number of patients, the extra laboratory time required for estimation of the bone markers, and the possibility of only 2D measurements of CBL. Future studies should be designed with larger sample sizes, longer observation periods, and the inclusion of chairside estimation of biomarkers. This may help establish a reference value for biomarkers for soft and hard tissue conditions around the implant. This may also help in the early assessment of homeostasis around the implant, as molecular changes will occur before the manifestation of clinical and radiographic changes.

**CONCLUSIONS**

Within the limitations of this clinical trial, the following conclusions were drawn:

1. OCN levels increased with time in both IL and DL implants.
2. IL implants showed a higher OCN level compared to DL implants over a period of 12 months.
3. CBL within physiologic limits takes place in all implants, irrespective of a loading protocol.

**ACKNOWLEDGMENTS**

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REFERENCES


