Randomised Controlled Trial
Dental Implants

The impact of different torques for the insertion of immediately loaded implants on the peri-implant levels of angiogenesis- and bone-related markers

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Abstract. The aim of this split-mouth, randomized, double-blind, controlled clinical trial was to evaluate the influence of different insertion torque values for dental implants on bone- and angiogenesis-related marker profiles. Eighteen edentulous patients received dental implants and fixed complete-arch mandibular prostheses. The implants (n = 36) were assigned randomly to two groups: reduced torque (n = 18), with insertion torque <30 N cm; and conventional torque (n = 18), with insertion torque ≥30 N cm. Levels of vascular endothelial growth factor (VEGF), placental growth factor, bone morphogenetic protein 9 (BMP-9), periostin, osteoprotegerin (OPG), and tartrate-resistant acid phosphatase (TRAP) in the peri-implant fluid were quantified at 7, 14, 30, and 120 days after implant placement. Inter-group comparisons showed that VEGF and OPG levels were higher in the low-level torque group than in the conventional torque group on days 7 and 30, respectively (P < 0.05). BMP-9 and peristatin levels were higher in the conventional group than in the low-level torque group on day 120, and TRAP was up-regulated around implants inserted with conventional torque when compared to those inserted with lower-level torque at all time points evaluated (P < 0.05). In conclusion, the use of different levels of torque for implantation of immediately loaded implants significantly influenced the levels of bone- and angiogenesis-related markers during early peri-implant repair.

Key words: torque; immediate dental implant loading; bone; biological markers; protein array.

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Treatment with dental implants is an established and predictable technique for the rehabilitation of partially and totally edentulous patients. The use of immediate- and early-loading implants has increased over the years, accelerating the function of the implants. Primary stability, defined as the absence of implant micromovements and directly influenced by the mechanical connection between the implant surface and the surrounding bone, has been described as essential for the success of dental implants, and is important to support early functional loading.

Experimental and clinical studies have attempted to identify a minimum insertion torque value to obtain adequate stability for immediate loading. It has been proposed that implants need to be inserted with a torque of at least 30 N cm for an immediate-loading protocol. In a clinical study on the immediate loading of single-tooth implants, Ottoni et al. established that immediate loading should only be proposed when the insertion torque is higher than 32 N cm. In line with this, Neugebauer et al. concluded that implants placed with an average insertion torque higher than 35 N cm were the most successful in minipigs.

However, there are conflicting reports in the literature regarding the consequences of using different levels of insertion torque during implant placement. From a biological perspective, some studies suggest that a higher insertion torque results in bone resorption before integration through bone formation. On the other hand, other studies have reported that lower insertion torques allow implant micromovement and connective tissue formation, leading to an absence of osseointegration. Furthermore, it has been reported that the use of a reduced insertion torque may result in empty spaces between the implant and the osteotomy site, which may lead to the formation of a blood clot immediately after implant placement and rapid replacement with woven bone, without prior bone resorption. Considering the results of these previous studies, it could be hypothesized that implants inserted with a reduced torque may promote a more promising pattern of bone repair when compared to those inserted with higher insertion torques, although this goes against the recommendations for insertion torque proposed in some studies.

Information in the literature regarding the peri-implant bone healing profile under different types of insertion torque is scarce, particularly for immediately loaded implants. In light of this, the objective of this study was to evaluate whether the use of different levels of insertion torque for immediately loaded dental implants could interfere with the levels of angiogenesis mediators and osteoblastogenesis and osteoclastogenesis-related factors in the peri-implant crevicular fluid during early peri-implant repair. These molecules are key markers of the host’s response and are thought to be critical mediators of bone repair.

Due to the emerging clinical demand for immediate implant loading, new evidence regarding the peri-implant angiogenesis- and bone-related biomarker profiles associated with immediately loaded implants inserted with different levels of torque would help to establish better surgical techniques for implant placement, thereby improving peri-implant repair. This in turn could contribute to improving the success of immediately loaded implants.

It was hypothesized that the use of different levels of torque for the insertion of immediately loaded dental implants could modulate the local pattern of bone vascular mediators during early bone healing around implants. Benefits in terms of the release of at least some bone and vascular mediators were expected using the reduced torque approach, i.e. the up-regulation of angiogenic factors (vascular endothelial growth factor (VEGF) and placental growth factor (PLGF)) and osteoblastic factors (bone morphogenetic protein 9 (BMP-9) and osteoprotegerin (OPG)), and the down-regulation of a marker of osteoclastogenesis (tartrate-resistant acid phosphatase (TRAP)).

Materials and methods

Patient population

The population of this prospective, split-mouth, randomized, double-blind, controlled clinical trial was recruited from patients referred to Paulista University between August 2014 and February 2016. The clinical procedures and evaluations were performed between October 2014 and March 2016. Data entry and statistical analyses were conducted by the end of October 2016. Eighteen patients were selected. Seven were male and 11 were female, and they ranged in age from 39 to 65 years. This study was approved by the university ethics committee.

Inclusion and exclusion criteria

The inclusion criteria for this study were as follows: patients with an edentulous mandibular arch indicated for rehabilitation with implants; any extractions performed at least 4 months before treatment; and age between 18 and 65 years.

The following exclusion criteria were applied: presence of systemic diseases that may interfere with bone repair (including diabetes, arthritis, hypothyroidism, hyperparathyroidism, and osteoporosis); the use of medications that would contraindicate the placement of implants or that are known to alter implant osseointegration (including anti-inflammatory and bisphosphonates) up to 6 months before surgery; bone grafts or a history of previous regenerative procedures in the area for implantation; insufficient bone for implant insertion; pregnant or breastfeeding women; and smokers or ex-smokers.

All eligible patients were provided with detailed information on the nature of the study and the potential benefits and risks of their participation, and they each signed an informed consent document.

Experimental groups

The selected patients received five immediately loaded implants between the mental foramina (single-stage dental implants) and a fixed implant-supported complete arch prosthesis. Using a computer-generated list (managed by F.R.C), two of the implants were assigned randomly to the following groups: reduced torque approach (n = 18), in which the implants were inserted with an insertion torque of <30 N cm; or conventional torque approach (n = 18), in which the implants were inserted with an insertion torque of ≥30 N cm (see Fig. 1 for the positions of the implants).

Implant therapy

The surgeries and all postoperative follow-up appointments were performed at the dental clinic of Paulista University, and all patients received five cylindrical dental implants (diameter 4.1 mm) with external hexagon connections (Implacil de Bortoli, São Paulo, Brazil) in the mandible. One surgeon (A.V.N) with clinical experience in implant dentistry performed all surgical procedures.

Briefly, the surgical area was anaesthetized, following which a crestal incision was made and a mucoperiosteal flap was raised. For the two experimental implants, careful site preparation was performed in order to obtain primary stability according to the insertion torque determined previously for each implant (<30 N cm or ≥30 N cm). The torque level was recorded by the drilling device (Chiropro; Bien-Air)
Dental SA, Bienne, Switzerland). For the implants inserted with the conventional torque approach, the surgical sequence followed the drilling protocol described by the implant company (single-stage dental implants). For the reduced torque approach implants, the surgical procedure was followed as described by the implant company, but over-preparation was performed (3.7 mm final twist drill). All patients were blinded to the implant group allocation.

Interrupted absorbable polyglyactin 910 sutures were placed (Vicryl; Ethicon, Somerville, NJ, USA). Amoxicillin (2 g administered 1 h before the procedure), dipyrone (500 mg every 6 h for 2 days postoperative), and 0.12% chlorhexidine mouthwash (every 12 h for 7 days) were prescribed.

All patients received Bränemark full-arch prostheses within 24 h of implant placement.

Evaluation of the angiogenesis- and bone-related marker profile by multiplex bead immunoassay (Luminex)

An examiner who was blinded to the implant allocation (R.A.) collected the peri-implant crevicular fluid from the implants in each group using filter paper strips (PerioPaper; Oraflow, Hewlett, NY, USA) at 7, 14, 30, and 120 days after implant insertion, as described previously. Briefly, the site was dried and isolated, following which an absorbent paper strip was inserted in the peri-implant sulcus until resistance was met. The strip was then left in place for 30 seconds. This procedure was performed at four sites per implant. The strips were then placed in separate tubes containing phosphate-buffered saline (PBS)/Tween. The fluid volume was measured using a calibrated device (Periotron 8000; Oraflow) and the peri-implant fluid samples were stored at $-80^\circ$C.

The levels of angiogenesis mediators (VEGF and PLGF), osteoblastogenesis-related factors (BMP-9, periostin, and OPG), and an osteoclastogenesis-related marker (TRAP) were determined using specific kits (HB NMAG–51 K, HRNKL MAG-31 K–01, and HAGP1 MAG–12 K; Millipore Corporation, Billerica, MA, USA) and a multiplex immunoassay instrument (MAgpix; MiraiBio, Alameda, CA, USA). The samples were analyzed individually, and the levels were estimated using a five-parameter polynomial curve (xPONENT software; Millipore Corporation). All results were adjusted for the volume of peri-implant crevicular fluid collected for each implant, and values were expressed in units of pg/ml.

Data analysis

The number of patients included in this study was based on previous investigations that have found significant differences in peri-implant and gingival crevicular fluid levels of various bone-related and immunoinflammatory markers.\[\text{16,17}\]

The data were initially analyzed for homogeneity using the Shapiro–Wilk test. Between-group comparisons of the data were then performed using analysis of variance (ANOVA) with the Tukey post-hoc test (bone/vascular markers) or the paired Student $t$-test (insertion torque).

All analyses were conducted using SAS 9.1 (SAS Institute Inc., Cary, NC, USA), considering each patient as an experimental unit and $\alpha = 5\%$.

Results

A total of 121 patients were initially selected, but 103 were excluded as they did not meet the inclusion criteria. Thus, 18 patients were included in the study; all of these patients completed the study and there were no drop-outs (Fig. 2). Of the 18 patients, 11 were female (61.1%) and seven were male (38.9%); their mean age was 58.06 ± 7.42 years (range 39–65 years). Measurements of insertion torque were averaged for both groups and are illustrated in Fig. 3. The average insertion torque in the low-level torque group was 19.18 ± 3.56 N cm and in the conventional torque group was 46.04 ± 7.48 N cm. A
Fig. 3. Insertion torque for the dental implants in each group; mean ± standard deviation values. *Statistically significant difference between the groups; paired Student t-test, \( P < 0.05 \).

statistically significant difference was observed between the groups, with higher insertion torque values in the conventional torque group \( (P < 0.05) \).

Levels of angiogenesis- and bone-related markers

The inter-group analysis showed higher VEGF and OPG levels in the low-level torque group when compared to the conventional torque group on days 7 and 30, respectively (both \( P < 0.05 \)) (Fig. 4A and B, respectively). In addition, the inter-group analysis showed significantly reduced levels of TRAP for implants with low-level insertion torque at all time points evaluated (7, 14, 30, and 120 days) when compared to those of the conventional insertion torque group (all \( P < 0.05 \); Fig. 4C). Conversely, regarding BMP-9 and periostin levels, both markers were increased in the conventional torque group compared to the low-level torque group at day 120 (both \( P < 0.05 \); Fig. 4D and E, respectively). Intra-group comparison revealed higher levels of PLGF at 120 days when compared to days 7 and 30 in the group with conventional insertion torque (both \( P < 0.05 \); Fig. 4F).

Discussion

The insertion torque is related to primary stability, which may interfere with the osseointegration process and consequently affect the success of immediately loaded implants. Although a minimum torque of 30 N cm has been proposed for an immediate-loading implant\(^{6–11}\), other studies have suggested that a greater insertion torque results in bone resorption before biological integration through bone formation, whereas lower torque could promote a more promising pattern of bone repair compared to higher insertion torques\(^{13–15,18}\). It appears that no clinical study to date has evaluated the impact of different levels of insertion torque on the pattern of peri-implant bone repair for immediately loaded implants. The present study investigated the levels of certain markers of osteoclastogenesis, osteoblastogenesis, and angiogenesis in the peri-implant fluid surrounding immediately loaded implants that had been inserted at different torques. In general, the results indicate that the torque applied affected the bone- and angiogenesis-related mediators released during early peri-implant repair.

Interestingly, the results revealed elevated concentrations of VEGF and OPG in the peri-implant fluid of implants inserted with a lower torque when compared to those inserted with conventional torque on days 7 and 30, respectively \( (P < 0.05) \). While VEGF is an important growth factor in the process of angiogenesis, related to chondrocyte \( \text{angiogenesis-related marker} \) and osteoblast differentiation\(^{19–21}\), OPG inhibits the differentiation and function of osteoclasts\(^{22}\), and these factors act to protect against bone loss.

In this trial, higher VEGF levels were observed at the beginning of bone repair (7 days) under the low-level insertion torque protocol when compared to the conventional torque protocol \( (P < 0.05; \text{Fig. 4A}) \). This outcome supports a probable beneficial effect of using a reduced torque during the surgical procedure for implant insertion, as this favours angiogenesis and the microcirculation. In agreement, animal studies have suggested that damage to the bone tissue and vessel obstruction leading to bone necrosis are related to the elevated pressure generated through the use of a high level of torque for implant insertion\(^{14,15,23}\).

Some in vitro investigations have demonstrated osteoblast differentiation by VEGF in a dose-dependent manner\(^{24}\), and additional evidence has confirmed the chemotactic effect of VEGF to human osteoblasts and mesenchymal progenitor cells\(^{25–26}\). Interestingly, considering the essential role of this angiogenesis marker in bone formation mechanisms, the increased level of VEGF observed in the initial stage (7 days) of repair in implants inserted with low-level torque in the present study may have positively influenced the subsequent up-regulation of OPG observed at 30 days when compared to the implants inserted with conventional torque \( (P < 0.05; \text{Fig. 4B}) \). This indicates the cooperation of angiogenic and osteogenic signalling to benefit peri-implant bone formation. OPG is an osteoblastogenesis-related marker that is important for preventing bone resorption. OPG binds to the receptor activator of nuclear factor kappa-B ligand (RANKL) to inhibit binding to its membrane receptor (RANK). Considering the favourable impact of VEGF and OPG during the bone remodelling process, the presence of higher levels of these mediators in the peri-implant fluid of implants inserted with low torque at the beginning of bone repair suggests a promising impact of this surgical technique in the modulation of key markers involved with osseous healing around implants.

Another outcome from the current investigation relates to the osteoclast-specific enzyme TRAP and suggests a positive impact of low insertion torque on the local host response in the area surrounding the implants. The data showed significantly reduced levels of TRAP around implants inserted under low torque when compared to those inserted with conventional torque at all time points evaluated (7, 14, 30, and 120 days) \( (P < 0.05; \text{Fig. 4C}) \). TRAP has long been established as a marker of osteoclast function\(^{27}\), and its activity is elevated in the serum of patients with bone disorders and harmful osseous repair\(^{28}\). Thus, the down-regulation of this marker in the peri-implant fluid of implants inserted with low torque indicates that it likely plays a role in the molecular events that occur during peri-implant healing. Conversely, the current trial revealed that even conventional torque values (average 46.04 N cm; Fig. 3) promoted the elevated release of TRAP throughout the study. This finding is in agreement with previous data demonstrating that the use of an insertion torque higher than 40–45 N cm may disturb the local microcirculation, leading to necrosis of the osteocytes and consequently to bone resorption\(^{29}\).

However, the current investigation revealed that implants inserted with conventional insertion torque showed favourable modulation of some
osteoblastogenesis-related markers at the final time point evaluated (120 days). The analysis showed that implants inserted with conventional torque promoted augmented release of BMP-9 at day 120 when compared to implants inserted with low torque \((P < 0.05; \text{Fig. 4D})\). BMPs are members of the transforming growth factor beta family and play important roles in the processes of bone formation and stem cell differentiation\(^3\). BMP-9 is one of the most highly osteogenic BMPs, and also promotes the differentiation of mesenchymal stem cells into osteoblasts\(^31,32\). The present study results showed that the greater torque promoted BMP-9 release, indicating osteoblastic activity during the late phase of osseointegration.

In agreement with these BMP-9 results, higher periostin levels were also observed at 120 days for the implants inserted with conventional torque when compared to those inserted with lower torque \((P < 0.05; \text{Fig. 4E})\). Periostin is a molecule expressed by osteocytes in bone that is capable of modulating Wnt-beta-catenin signalling. The levels of periostin mRNA
and protein have been shown to be up-regulated during fracture healing, particularly in proliferating osteoblast cells. It is speculated that periostin plays a role in the recruitment of cells of the osteoblastic lineage to the site of repair.

Importantly, periostin shows higher expression under mechanical stress and tension. In a study by Bonnet et al., periostin mRNA and protein were found to be overexpressed in the mouse tibia under axial compression. This suggests that up-regulation of this osteoblastic factor in implants inserted with higher torque could be related to the augmented loading at the bone-implant interface.

Another relevant finding in this study is related to the increased levels of PLGF, an important angiogenic-related factor involved in bone formation and repair processes. For implants inserted with conventional torque, PLGF was higher in the late stage of repair (120 days) when compared to the earlier stages (7 and 30 days) (P < 0.05; Fig. 4F). Interestingly, an in vitro study by McCoy et al. demonstrated that PLGF is a mechanosensitive, marker of which is proportional to both the magnitude and duration of the mechanical stimulus applied. The elevated peri-implant levels of PLGF in the implants inserted with conventional torque at the final time point (120 days) may also be in response to the augmented load at the bone–implant interface, in agreement with the up-regulation of periostin in this experimental group at the same time point.

Some studies have tried to establish the ideal insertion torque to improve bone healing during immediate-loading rehabilitation. An experimental study in dogs by Rea et al. showed that a higher mineralized bone-to-implant contact was observed when a low torque (approximately 30 N·cm) was used compared with a high insertion torque (>70 N·cm) in immediately loaded implants. In line with this, Testori et al. stated that a minimum insertion torque of 30 N·cm is required for successful immediate-loading protocols from a clinical point of view. Nevertheless, from a molecular point of view, the biochemical data from the peri-implant fluid analysed in the present study demonstrated that a reduced torque approach (average insertion of 19.18 N·cm) provided additional benefits in terms of the release of bone and vascular mediators when compared to the conventional torque approach (average insertion of 46.04 N·cm). When combined, the findings of this study suggest that osteoblastic activity may predominantly be observed following insertion with low-level torque, especially during the first days of bone repair after implantation, considering the up-regulation of VEGF and OPG in the peri-implant fluid seen during this period. On the other hand, data from the analyses demonstrated that conventional-level torque was related to up-regulated BMP-9 and periostin at the final evaluation time point, suggesting an osteoblastic influence only during late peri-implant repair, whereas osteoclastic events were predominant when the higher insertion force was used during surgical implant placement. While these relevant molecular findings may highlight the mechanisms involved in peri-implant bone repair in the earlier stage of osseointegration after the implantation of immediate-loading implants, further studies are necessary to determine precise clinical guidelines.

The preliminary outcomes from this investigation suggest some biological mechanisms that could explain the positive effects of lower insertion torque during initial peri-implant healing. The results of this study provide a basis for future studies on the possible relevance of the surgical technique for implant placement with low or high insertion torque, and to better understand the bone and vascular changes around the implants. This information could be used to determine the best treatment strategy and method of osteotomy preparation to benefit implant loading and lead to quicker prosthesis rehabilitation.

Considering the points discussed, the results of this study confirm the suggested hypothesis that different levels of torque for the insertion of dental implants may modulate the release of angiogenesis- and bone-related markers.

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Competing interests
Nothing to declare.

Ethical approval
This study was approved by the university ethics committee (44161215.3.0000.5512).

Patient consent
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References


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