

# Biochemical marker levels in peri-implant crevicular fluid of completely edentulous patients with two implant-supported mandibular overdentures before and 6 months after loading: Prospective clinical trial

Nazlı Zeynep Alpaslan Yaylı<sup>1</sup>, Eylem Ayhan Alkan<sup>2</sup>

<sup>1</sup> Van Yüzüncü Yıl University, Faculty of Dentistry, Department of Periodontology, Van Turkey

<sup>2</sup> Ankara Yıldırım Beyazıt University, Faculty of Dentistry, Department of Periodontology, Ankara, Turkey

## Abstract

**Aim:** The aim of this study was to determine the levels of biochemical markers detected in the peri-implant crevicular fluid (PICF) before and 6 months after loading in completely edentulous patients with two implant-supported mandibular overdentures and to correlate them with clinical parameters.

**Methodology:** Two implants were applied to the mandibular canine region of the 20 completely edentulous and systemically healthy patients (11 females, 9 males) with conventional 2-stage surgery. PICF samples of the participants were collected before and 6 months after prosthetic loading, and clinical parameters—including modified plaque index (MPI), gingival index (GI), bleeding on probing (BoP), probing pocket depth (PPD), and keratinized gingival width (KGW)—were recorded. Interleukin-18 (IL-18), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), matrix metalloproteinase-8 (MMP-8), and aspartate aminotransferase (AST) levels were examined in PICF samples via enzyme-linked immunosorbent assay (ELISA).

**Results:** The average age of the participants was  $57.05 \pm 9.82$  years. Although a decrease was observed in all clinical parameters after prosthetic loading compared to baseline levels, this decrease was not considered statistically significant ( $p > 0.05$ ). PICF volume showed a statistically significant decrease from baseline to 6 months ( $p < 0.05$ ). The decreases observed at 6<sup>th</sup> month values in all of the biochemical markers examined were not found to be statistically significant, similar to the clinical parameters ( $p > 0.05$ ).

**Conclusion:** Within the limits of this study, the PICF marker levels and clinical parameters determined following osseointegration and 6 months after prosthetic loading are similar. This suggests that the studied sample has prosthetic restorations that contribute positively to the tissue maturation process and apply ideal forces to the tissue. More comprehensive studies are recommended to understand the tissue healing process after implant treatment.

**Keywords:** dental implant, two-stage surgery, peri-implant crevicular fluid, biochemical marker

## Correspondence:

Dr. Nazlı Zeynep ALPASLAN YAYLI  
Van Yüzüncü Yıl University,  
Faculty of Dentistry, Department  
of Periodontology, Van, Turkey.  
E-mail: [nzeypnep\\_5@hotmail.com](mailto:nzeynep_5@hotmail.com)

Received: 11 March 2021

Accepted: 18 May 2021

## Access Online



DOI:

10.5577/intdentres.2021.vol11.suppl1.24

**How to cite this article:** Alpaslan Yaylı NZ, Ayhan Alkan E. Biochemical marker levels in peri-implant crevicular fluid of completely edentulous patients with two implant-supported mandibular overdentures before and 6 months after loading: Prospective clinical trial. Int Dent Res 2021;11(Suppl.1):160-6. <https://doi.org/10.5577/intdentres.2021.vol11.suppl1.24>

## Introduction

Implant treatment has become one of the first options for prosthetic rehabilitation of fully edentulous and partially edentulous jaws (1). The use of implants, especially for the stabilization of mandibular full dentures, is among the greatest achievements of modern dentistry. It is well documented in the literature that stabilization of lower jaw prostheses with 2 implants reduces peri-implant bone atrophy, increases chewing efficiency, decreases masseter muscle atrophy, and significantly improves the patient's oral health-related quality of life (2).

The original Branemark concept consists of a 2-part dental implant designed for use in a 2-stage treatment procedure. In this concept, after the soft tissue flap is elevated, the implant is placed in the bone, and the flap is repositioned so that the implant remains closed during healing. Following the healing period, the flap is elevated again, and a transmucosal abutment is inserted for prosthetic attachment (3).

Establishing a direct structural and functional connection between living bone and the surface of a load-bearing implant is defined as osseointegration and is essential for implant success. Understanding the biological interactions between the implant and the surrounding bone is very important. The importance of the advanced assessment techniques required to understand and verify osseointegration is highlighted (4).

As a result of surgical trauma during implant placement, both bone and soft tissue wounds occur (5). Dynamic processes that result in the integration of the implant with bone and the formation of a connecting apparatus include inflammation mediated by activated resident or migrating cells, matrix deposition, bone resorption and apposition, and initiation and eventual dissolution of neurogenesis and angiogenesis (6, 7). In a pilot animal study, Trindade et al. recently found evidence that the immune system plays a role in the osseointegration process around titanium implants (8). It is stated that the interactions between cells and related biological stages required for correct healing and tissue homeostasis are modulated by cytokines, chemokines, and growth factors (9). Cytokines control cell line interactions during the proliferative phase in hard tissue. Cytokine-mediated cell activation leads to increased synthesis of the non-collagenous and collagenous protein structures required for the formation of the organic matrix through cytokine-mediated signal transduction and transcription (4).

Maintaining a long-term balance between host cells and titanium is crucial to the success of implants (10). Understanding and monitoring the profiles of cytokines secreted by macrophages may be important for systematically analyzing and predicting the performance of implants (11). Biomarkers such as cytokines, proteins, and multifunctional peptides act as intercellular regulatory factors found locally and systemically in peri-implant crevicular fluid (PICF). These biomarkers modulate the intensity of inflammation, foreign body reaction, cellular organization, healing, and disease pathogenesis (12).

Along with osseointegration and pathological processes, there is little evidence concerning which biomarkers play a role in PICF in maintaining the bio-equilibrium state. Understanding the cytokine mechanisms that function during osseointegration and remodeling processes will improve the ability of researchers to determine the prognosis of implants and allow early diagnosis of peri-implant diseases that may develop based on patient sensitivity. Additionally, conducting clinical studies investigating biomarkers in PICF prior to implant loading may allow the identification of potential confounding factors, such as host immuno-response capacity, that may impair implant healing. In light of all this information, the aim of the study is to determine the levels of interleukin-1B (IL-1B), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), matrix metalloproteinase-8 (MMP-8), and aspartate aminotransferase (AST) detected before and 6 months after prosthetic loading in the PICF of fully edentulous patients with 2 implant-supported lower jaw overdentures and to correlate these values with clinical parameters.

## Materials and Methods

### Study Population and Study Design

This study was conducted on 20 fully edentulous patients (11 females and 9 males) who presented to the Van Yüzüncü Yıl University (YYU) Faculty of Dentistry, Department of Prosthodontics with complaints about the retention and stability of their prostheses; these patients were then referred to the Department of Periodontology. Approval was obtained from the Clinical Research Ethics Committee of YYU's Faculty of Medicine for the study (10.01.2014/05). The patients were informed in detail about the study, and written informed consent was obtained from each participant. Later, a total of 40 same-brand sandblasted large-grit acid-etched (SLA) surface implants (Implant Direct, Sybron International, LLC., Calabasas Hills, California, USA) were applied to the mandibular canine region (two for each patient), with a 2-stage surgical procedure. All procedures were performed by the same clinician.

#### Inclusion criteria determined as;

- 1) Patients' volunteering for the study,
- 2) Being completely edentulous individuals,
- 3) They do not have a systemic operation risk such as diabetes, hematological/metabolic diseases, use of bisphosphonates,
- 4) They have not received radiotherapy from the head and neck region,
- 5) No alcohol or drug addiction,
- 6) Being non-smokers,
- 7) The absence of any clinically and radiologically detected pathologies in the jaw bones,
- 8) The tooth extraction in the area where the implant is planned to be placed has been performed 6 months or before, and there is sufficient bone support in the relevant area,
- 9) No need for a further surgical procedure such as block bone graft placement for implant operation,

- 10) Absence of pregnancy or breastfeeding,
- 11) Not having received antibiotic treatment for the last 6 months.

### Implant Placement Surgery

Panoramic radiographs and, if necessary, dental tomography images were obtained from the participants who met the inclusion criteria before the implant operation. After local infiltrative anesthesia was applied to the operation area, crestal incisions were made over the alveolar crest with a scalpel no.15, and additional vertical incisions were made when necessary. After the incisions, full-thickness mucoperiosteal flaps were elevated with the periosteum elevator, and any soft tissue remaining on the crest was removed. The placement of implants in appropriate sizes for pre-surgical examinations were carried out in accordance with the protocol recommended by the manufacturer (Implant Direct, Sybron International, LLC., Calabasas Hills, California, USA). After the osteotomy procedures, the implants taken with the carrier piece were placed in the bone at the depth of the osteotomy. After the closure screws were attached, the area was closed primarily with silk sutures. After the procedure, analgesic tablets (Apranax Fort 550 mg, Abdi İbrahim Pharm. Ind. and Trd. Comp., Istanbul, Turkey, 2x1) and disinfectant mouthwash (Klorhex 0.2%, Drog-San Pharm. Ind. and Trd. Comp., Ankara, Turkey) were prescribed to the patients, and the sutures were removed 7-10 days later.

### Second Stage Surgery

Patients who underwent implantation were invited to the 3<sup>rd</sup> month control and the second surgery was performed under local anesthesia, and the closure screws were removed. Healing caps were attached to the patients with no mobility in the implant, no pain, numbness, or infection in the relevant area, no radiolucency or bone loss around the implant on the radiography, and osseointegration was achieved. Then the area was covered with silk sutures again. After 7-10 days, the sutures were removed, and an appointment was made for two weeks later to ensure gingival healing.

### Measurement of Clinical Parameters

The modified plaque index (MPI) of patients whose gingival healing was completed was measured before PICF was collected to prevent impairment of plaque formation (13). Other clinical parameters, including the gingival index (GI) (13), probing pocket depth (PPD), bleeding on probing (BoP) (14), and keratinized gingival width (KGW) (the distance from the marginal gingiva to the mucogingival line) were measured after PICF was collected. MPI, GI, PPD, and BoP values were recorded and averaged by the same clinician from 4 surfaces of implants (mesial, buccal, distal, and lingual), and KGW values were measured from the midbuccal region under gentle forces using a Williams-type plastic periodontal probe (Premier, PerioWise, Premier Products Co., North America).

### Collection of Peri-implant Crevicular Fluid Samples

Prior to PICF collection, the site was isolated from saliva with cotton rolls. The microbial dental plaque on the implants was removed, and the area was dried by gently squeezing air. Paper strips specially produced for PICF collection (PerioPaper™ GCF Collection Strips, Oraflow Inc., Smithtown, New York, USA) were inserted 1-2 mm into the implant groove with a tweezers and kept for 30 seconds. During sampling, care was taken to avoid bleeding and to prevent contamination of the area with saliva. PICF samples were collected from three surfaces of each implant: mesial, distal, and buccal. The paper strips were then placed in a nearby Periotron (Periotron® 8000-220 Volt, Oraflow Inc., Smithtown, New York, USA) device to measure PICF volumes. PICF collected paper strips are placed in sterile eppendorf tubes (SealRite® 1.5 ml Microcentrifuge Tubes, USA Scientific, Inc., Orlando, Florida) containing 500 µl PBS (phosphate-buffered saline, pH: 7.0) solution and stored at -20 C° until analysis.

The patients were given an appointment for the 6th month control after the prosthetic loading, and they were directed to the Department of Prosthetic Dentistry for their prosthesis. Clinical data of the patients who came for the 6th month follow-up visit were re-recorded, and PICF samples were recollected. Oral hygiene training was repeated for the necessary participants.

### Laboratory Analysis

For analysis of total interleukin-1B (IL-1B), tumor necrosis factor-α (TNF-α), matrix metalloproteinase-8 (MMP-8) and aspartate aminotransferase (AST) levels in PICF samples, samples stored at -20 C° were thawed, centrifuged, vortexed and then placed in 96-well plates with a graduated micropipette. Analysis of the samples was done with the Enzyme-Linked Immunosorbent Assay (ELISA). ELISA test was performed in accordance with the manufacturer's recommended protocol (Novex® by life technologies™ Invitrogen™ Human IL-1B ELISA Kit, Thermo Fisher Scientific Inc. Camarillo, California; Novex® by life technologies™ Invitrogen™ Human TNF-α ELISA Kit, Thermo Fisher Scientific Inc. Camarillo, California; RayBio® Human MMP-8 ELISA Kit RayBiotech, Inc. Norcross, GA; USCN Human AST ELISA Kit USCN Life Science Inc. Wuhan, Hubei, China, respectively). Absorbance values were read with an ELISA reader (µQuant™ ELISA Microplate Reader, BioTek® Instruments, Inc., Vermont, USA) at 450 nm wavelength. Since there are three paper strips in each Eppendorf tube, the amount of cytokine and enzyme determined in the laboratory was divided into three and the total value of the relevant marker was calculated. The PICF volumes measured in the Periotron® device were also divided by 3, and the PICF volume for each implant was calculated.

## Statistical analysis

Descriptive statistics for the features emphasized is expressed as mean, standard deviation, minimum and maximum value. Pairing t-test was used to compare the baseline and sixth month values. Pearson correlation coefficients were calculated to determine the relationship between variables. The statistical significance level was taken as 5% in the calculations, and the “the statistical package for the social sciences 22.00 (SPSS)” package program was used for calculations (IBM Corp, Armonk, NY, USA).

## Results

11 of the participants were women, and 9 of them were men, and the average age was  $57.05 \pm 9.82$ . The

statistical analyzes of the initial (before loading) and 6<sup>th</sup> month (6<sup>th</sup> month after loading) values of the clinical parameters of the patients are shown in Table 1. Although a decrease was observed in all clinical parameters examined in the 6<sup>th</sup> month after prosthetic loading compared to the baseline levels, this decrease was not considered statistically significant ( $p>0.05$ ). Statistical analysis of the data of the initial and 6<sup>th</sup> month PICF samples of the participants is shown in Table 2. Clinically measured PICF volume was  $96.638 \pm 33.438$  at baseline and  $76.044 \pm 34.289$  at 6 months. The decrease in the value obtained at 6 months for the PICF volume compared to the baseline value was statistically significant ( $p<0.05$ ). Decreases in the total values of IL-1 $\beta$ , TNF- $\alpha$ , MMP-8 and AST biomarkers were detected in the 6<sup>th</sup> month compared to the baseline levels, but these decreases were not statistically significant ( $p>0.05$ ).

**Table 1.** Statistical evaluations of clinical parameters obtained before and 6 months after prosthetic loading

Clinical Parameters	Before Prosthetic Loading			6 Months After Prosthetic Loading			p
	Mean $\pm$ SD	Min.	Max.	Mean $\pm$ SD	Min.	Max.	
MPI (score)	0.425 $\pm$ 0.501	0	1	0.25 $\pm$ 0.439	0	1	0.1
GI (score)	1.031 $\pm$ 0.129	0.5	1.25	0.981 $\pm$ 0.164	0.5	1.5	0.134
BoP %	5 $\pm$ 10.127	0	25	1.875 $\pm$ 8.748	0	50	0.144
PPD (mm)	1.813 $\pm$ 0.69	1	3	1.644 $\pm$ 0.725	1	3	0.29
KGW (mm)	2.075 $\pm$ 2.28	0	7	2.05 $\pm$ 2.241	0	7	0.961

\* $p<0.05$

SD: Standard deviation, Min.: Minimum, Max.: Maximum, MPI: Modified plaque index, GI: Gingival index, BoP: Bleeding on probing, PPD: Probing pocket depth, KGW: Keratinized gingival width, mm: Millimeter, %: Percentage

**Table 2.** Statistical evaluations of PICF data obtained before and 6 months after prosthetic loading

Biochemical Parameters	Before Prosthetic Loading			6 Months After Prosthetic Loading			p
	Mean $\pm$ SD	Min.	Max.	Mean $\pm$ SD	Min.	Max.	
PICF Volume $\mu$ l / 30 sec	96.638 $\pm$ 33.438	48.8	185	76.044 $\pm$ 34.289	30.8	169	0.008*
IL-1 $\beta$ Total Value pg / 30 sec	0.183 $\pm$ 0.333	0.01	1.55	0.11 $\pm$ 0.127	0.01	0.52	0.198
TNF- $\alpha$ Total Value pg / 30 sec	0.465 $\pm$ 0.495	0.06	2.02	0.462 $\pm$ 0.447	0.07	1.47	0.979
MMP-8 Total Value pg / 30 sec	1.321 $\pm$ 0.73	0.07	2.83	1.078 $\pm$ 0.479	0.06	1.73	0.082

AST Total Value ng / 30 sec	0.664±0.443	0.08	1.81	0.58±0.499	0.03	2.29	0.429
--------------------------------	-------------	------	------	------------	------	------	-------

\*p<0.05

SD: Standard deviation, Min.: Minimum, Max.: Maximum, PICF: Peri-implant crevicular fluid, IL-1B: Interleukin-1B, TNF-α: Tumor necrosis factor-α, MMP-8: Matrix metalloproteinase-8, AST: Aspartate aminotransferase, µl: Microliter, sec: Second, pg: Picogram, ng: Nanogram

## Discussion

Current research of dental implant applications in dentistry focuses on understanding the influence of various factors on osseointegration. This prospective clinical study was conducted to determine the levels of IL-1B, TNF-α, MMP-8, and AST biomarkers detected in the PICF of fully edentulous patients with two implant-supported mandibular overdentures before and 6 months after prosthetic loading and to correlate them with clinical parameters. The results of the study showed insignificant decreases in all clinical and biochemical data except PICF volume, which showed a significant decrease in 6 months values.

It is stated that traditional two-piece implants will usually function after 3 to 6 months of healing (15). Based on this information, the clinical and biochemical parameters evaluated in the present study were examined following osseointegration and after 6 months of prosthesis use.

The correlation between clinical indicators of peri-implant health monitoring and marginal bone loss is currently being questioned. Common periodontal evaluations such as bleeding on probing and probing depth are not always a reliable tool for assessing the health of marginal soft and hard tissue in the peri-implant area (16,17). Biochemical analysis of gingival crevicular fluid (GFC) is performed in addition to standard clinical tests in order to evaluate the inflammatory status of the gingival tissues and to improve the methodology. Collecting the crevicular fluid enables the measurement of biomarkers important for periodontal disease because biomarkers are secreted products of immune cells and represent the innate immune response to bacterial pathogens and danger signals (18). Considering the effects of host response and immunity on the tissue healing process (7), in this study, both clinical and molecular evaluations were made during the maturation of the hard and soft tissue around the implants. It has been reported in the literature that various biomarkers such as IL-1B, IL-8, TNF-α, MMP-8, tissue inhibitors of metalloproteinases-1 (TIMP-1), and Prostaglandin E2 (PGE2) are active in conditions involving wound healing and pathological processes, and they can be analyzed within the PICF and can provide useful evidence (12). Only one study has been found showing that AST has been examined within the PICF (19). Since both clinical parameters including modified plaque index (MPI), gingival index (GI), bleeding on probing (BoP), probing pocket depth (PPD), keratinized gingival width (KGW),

and IL-1B, TNF-α, MMP-8, AST biomarkers in PICF have been studied depending on the follow-up time in our study, it is thought that it will contribute to the literature.

Long-term maintenance and success of implants include remodeling activity that continues around the implant. Remodeling provides prevention of fracture due to bone fatigue and replacement of bone that may have permanent micro-cracks as a result of cyclic loading (20). The scarcity of clinical studies conducted during remodeling and tissue maturation made it difficult to compare research results. Biomarkers of IL-1B, TNF-α, MMP-8, and AST examined in this study were detected both at baseline and 6 months after loading but varied depending on time. The presence and changes of various biomolecules in the early and later stages of healing indicate that the establishment and maintenance of osseointegration and the formation of a peri-implant mucosal seal are dynamic biological processes governed by molecular signals (9, 21). The reductions detected at both clinical and molecular levels for the parameters examined in the 6<sup>th</sup> month, partially comply with the findings of some previous studies (6, 22). The authors stated that mucosal inflammation, which is a determinant in the healing of the peri-implant mucosa, persists until the 12th week, and they emphasized that the biomarker levels, which were initially high, as an indicator of an acute response due to surgical trauma, remained lower in the stages when late remodeling is completed, and the epithelium and connective tissue mature. In our study, all clinical and biochemical data, except PICF volume, which showed a significant decrease, insignificant reductions have been detected in the 6th month following loading. This result partially complies with the literature. The decreases observed in the parameters indicate that tissue maturation is at a more successful level than at the beginning. Initial measurements were made after the completion of the gingival healing process after the second surgery and ≈ 3.5 months after implant placement. However, considering the initial clinical parameter values, it is observed that the peri-implant area is relatively healthy, and the oral hygiene level is at an acceptable level. Biochemical marker levels are likely to be detected at much higher levels in the earlier stages of tissue healing. This may explain the lack of significant difference in values measured at two-time points. When immediate and early loading procedures are to be performed, it is thought that conducting analyzes to determine the host response of the individuals and their current biomarker levels may

prevent early implant failure. Because although well-established surgical implant protocols achieve a  $\geq 95\%$  success rate, early failures are still a major concern among clinicians and researchers. These early failures occur without a known biological mechanism, and there appears to be no evidence that primary infection is the main causal factor for marginal bone resorption (11, 12).

While examining the soft tissue around the implant, the literature points out that the presence of sufficient keratinized gingival width (KGW) is important in terms of aesthetics, function, and long-term implant stability (23). In a meta-analysis (24), it is stated that there are contradictory results in terms of the effects of KGW on implant stability, but the general opinion is that less plaque accumulation, less inflammation, and less mucosal recession can be seen in the presence of sufficient KGW. In this research, it is seen that the average KGW of the participants is  $\approx 2$  mm. Considering the 6th month clinical and biochemical data of the study, a statistically insignificant decrease compared to the baseline level indicates that sufficient KGW has a positive contribution to the tissue maturation process and implant stability. In addition, the control of plaque accumulation throughout the study and the presence of minimal inflammation in the peri-implant site support this information.

Coarse sandblasted and then acid etched pure titanium improves the mechanical clamping between the bone and the titanium implant, which increases the shear strength. In addition, it has been proven that implant surface topography has a significant effect on osteoblast proliferation and differentiation (4). However, it has been shown that the mechanical load will play an important role in the development, maintenance, and adaptation of the skeleton (25). In an animal study by Berglundh et al. (26), it was reported that implants exposed to a functional load exhibited a higher degree of bone-implant contact and that functional load could increase osseointegration and did not cause marginal bone loss. In our study, the results obtained in the 6th month were more successful than the initial values, suggesting that the same brand of SLA-surfaced implants applied to the study population and prosthetic restorations used in the upper structure apply functional forces to the tissues within physiological limits and have a positive effect on the healing process.

The main limitations of this study were that it was a single-center study with a limited number of samples, the biomarker levels in earlier healing stages could not be detected since individuals who underwent 2-stage surgery were included, and the peri-implant mucosal phenotype, which is a critical parameter for tissue maturation, was not examined. More comprehensive studies are needed to evaluate all these parameters in order to better understand the processes of wound healing, tissue maturation, and remodeling after implant treatment.

## Conclusions

This research examined the levels of IL-1 $\beta$ , TNF- $\alpha$ , MMP-8, and AST biomarkers in PICF during the recovery period after implant placement (immediately after osseointegration and 6 months after prosthetic loading) and increased the available data due to the limited number of clinical studies conducted in this area. In all clinical and biochemical data except PICF volume, which showed a significant decrease in 6 months after prosthetic loading, insignificant decreases were detected. These findings are thought to be related to the resolution of inflammation, the formation of the mucosal barrier, the sufficient keratinized gingival width of the research population, and the presence of prosthetic superstructures exerting ideal forces at physiological limits.

**Acknowledgments:** This study was presented as a full-text oral presentation at the 1<sup>st</sup> International Dental Research and Health Sciences Congress held between 20-22 May 2021.

**Ethical Approval:** Ethics committee approval was received for this study from Van Yüzüncü Yıl University, Faculty of Medicine in accordance the World Medical Association Declaration of Helsinki, with the approval number: 10.01.2014/05.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Conception - N.Z.A.Y.; Design - N.Z.A.Y., E.A.A.; Supervision - N.Z.A.Y.; Materials - N.Z.A.Y., E.A.A.; Data Collection and/or Processing -N.Z.A.Y.; Analysis and/or Interpretation - N.Z.A.Y., E.A.A.; Literature Review - N.Z.A.Y.; Writer - N.Z.A.Y.; Critical Review - N.Z.A.Y., E.A.A.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** This study has been supported by the Scientific Research Projects Coordination Unit of Van Yüzüncü Yıl University, Turkey. The authors would like to thank for their valuable support for the conduct of the study (Project No: 2014-SBE-D045).

## References

1. Jimbo R, Albrektsson T. Long-term clinical success of minimally and moderately rough oral implants: a review of 71 studies with 5 years or more of follow-up. *Implant Dent* 2015;24(1):62-9. ([Crossref](#))
2. Schimmel M, Müller F, Suter V, Buser D. Implants for elderly patients. *Periodontol* 2000 2017;73(1):228-40. ([Crossref](#))
3. Prithviraj DR, Gupta V, Muley N, Sandhu P. One-piece implants: placement timing, surgical technique, loading protocol, and marginal bone loss. *J Prosthodont* 2013;22(3):237-44. ([Crossref](#))
4. von Wilmsky C, Moest T, Nkenke E, Stelzle F, Schlegel KA. Implants in bone: part I. a current overview about tissue response, surface modifications and future perspectives. *Oral Maxillofac Surg* 2014;18(3):243-57. ([Crossref](#))
5. Trindade R, Albrektsson T, Tengvall P, Wennerberg A. Foreign body reaction to biomaterials: on mechanisms for buildup and breakdown of osseointegration. *Clin Implant Dent Relat Res* 2016;18(1):192-203. ([Crossref](#))
6. Gürkan A, Tekdal GP, Bostancı N, Belibasakis GN. Cytokine, chemokine, and growth factor levels in peri-implant sulcus

- during wound healing and osseointegration after piezosurgical versus conventional implant site preparation: Randomized, controlled, split-mouth trial. *J Periodontol* 2019;90(6):616-26. ([Crossref](#))
7. Palmquist A, Omar OM, Esposito M, Lausmaa J, Thomsen P. Titanium oral implants: surface characteristics, interface biology and clinical outcome. *J R Soc Interface* 2010;7(5):515-27. ([Crossref](#))
  8. Trindade R, Albrektsson T, Galli S, Prgomet Z, Tengvall P, Wennerberg A. Osseointegration and foreign body reaction: titanium implants activate the immune system and suppress bone resorption during the first 4 weeks after implantation. *Clin Implant Dent Relat Res* 2018;20(1):82-91. ([Crossref](#))
  9. Shanbhag S, Shanbhag V, Stavropoulos A. Genomic analyses of early peri-implant bone healing in humans: a systematic review. *Int J Implant Dent* 2015;1(1):5. ([Crossref](#))
  10. Albrektsson T, Dahlin C, Jemt T, Sennerby L, Turri A, Wennerberg A. Is marginal bone loss around oral implants the result of a provoked foreign body reaction? *Clin Implant Dent Relat Res* 2014;16(2):155-65. ([Crossref](#))
  11. Ma QL, Fang L, Jiang N, et al. Bone mesenchymal stem cell secretion of sRANKL/OPG/M-CSF in response to macrophage-mediated inflammatory response influences osteogenesis on nanostructured Ti surfaces. *Biomaterials* 2018;154:234-47. ([Crossref](#))
  12. Bielemann AM, Marcello-Machado RM, Del Bel Cury AA, Faot F. Systematic review of wound healing biomarkers in peri-implant crevicular fluid during osseointegration. *Arch Oral Biol* 2018;89:107-28. ([Crossref](#))
  13. Mombelli A, van Oosten MA, Schurch E Jr, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiol Immunol* 1987;2(4):145-51. ([Crossref](#))
  14. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J* 1975;25(4):229-35.
  15. Cannizzaro G, Leone M, Esposito M. Immediate versus early loading of two implants placed with a flapless technique supporting mandibular bar-retained overdentures: a single-blinded, randomised controlled clinical trial. *Eur J Oral Implantol* 2008;1(1):33-43.
  16. Albrektsson T, Buser D, Sennerby L. Crestal bone loss and oral implants. *Clin Implant Dent Relat Res* 2012;14(6):783-91. ([Crossref](#))
  17. Coli P, Christiaens V, Sennerby L, Bruyn H. Reliability of periodontal diagnostic tools for monitoring peri-implant health and disease. *Periodontol 2000* 2017;73(1):203-17. ([Crossref](#))
  18. Bostanci N, Belibasakis GN. Gingival crevicular fluid and its immune mediators in the proteomic era. *Periodontol 2000* 2018;76(1):68-84. ([Crossref](#))
  19. D'Ercole S, Tetè S, Catamo G, et al. Microbiological and biochemical effectiveness of an antiseptic gel on the bacterial contamination of the inner space of dental implants: a 3-month human longitudinal study. *Int J Immunopathol Pharmacol* 2009;22(4):1019-26. ([Crossref](#))
  20. Ghanavati F, Shayegh SS, Rahimi H, et al. The effects of loading time on osseointegration and new bone formation around dental implants: a histologic and histomorphometric study in dogs. *J Periodontol* 2006;77(10):1701-7. ([Crossref](#))
  21. Insua A, Monje A, Wang HL, Miron RJ. Basis of bone metabolism around dental implants during osseointegration and peri-implant bone loss. *J Biomed Mater Res A* 2017;105(7):2075-89. ([Crossref](#))
  22. Tomasi C, Tessarolo F, Caola I, et al. Early healing of peri-implant mucosa in man. *J Clin Periodontol* 2016;43(10):816-24. ([Crossref](#))
  23. Lin GH, Chan HL, Wang HL. Effects of currently available surgical and restorative interventions on reducing midfacial mucosal recession of immediately placed single-tooth implants: a systematic review. *J Periodontol* 2014;85(1):92-102. ([Crossref](#))
  24. Lin CY, Chen Z, Pan WL, Wang HL. Impact of timing on soft tissue augmentation during implant treatment: a systematic review and meta-analysis. *Clin Oral Implants Res* 2018;29(5):508-21. ([Crossref](#))
  25. Tanck E, Homminga J, van Lenthe GH, Huijkes R. Increase in bone volume fraction precedes architectural adaptation in growing bone. *Bone* 2001;28(6):650-4. ([Crossref](#))
  26. Berglundh T, Abrahamsson I, Lindhe J. Bone reactions to longstanding functional load at implants: an experimental study in dogs. *J Clin Periodontol* 2005;32(9):925-32. ([Crossref](#))