RESEARCH

Effect of anatomical location (mandible vs maxilla) of dental implants on the BMP-2, BMP-7, sRANKL and OPG levels in peri-implant crevicular fluid during osseointegration. A pilot study

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ABSTRACT

Effect of anatomical location (mandible vs maxilla) of dental implants on the BMP-2, BMP-7, sRANKL and OPG levels in peri-implant crevicular fluid during osseointegration. A pilot study

Background: The aim of this study was to investigate levels of bone morphogenetic protein-2 (BMP-2), BMP-7, soluble receptor activator of nuclear factor-kB ligand (sRANKL) and osteoprotegerin (OPG) in the peri-implant crevicular fluid (PICF) of implants placed in both maxilla and mandible during the osseointegration period.

Materials and Methods: Thirty-three patients (17 females and 16 males; mean age 47.03±11.23 years) were included in this study. A total of 33 implants were placed in both of maxilla (Group 1/n=18) and mandible (group 2/n=15). Peri-implant crevicular fluid (PICF) samples, modified plaque index (MPI), gingival index (GI) and probing depth (PD) measurements were obtained at 1 and 3 months after surgery. PICF levels of BMP-2/-7, sRANKL and OPG were analyzed by ELISA.

Results: No complications were observed during the healing period. No significant differences were observed in the PICF levels of sRANKL, OPG, BMP-2 and BMP-7 and evaluated clinical parameters between groups at any time point ($p>0.05$). While PICF volume of group 2 was greater than group 1 at first month, PICF volume of group 1 was greater than group 2 at 3 months ($p<0.05$). There was a positive correlation between sRANKL levels and PICF volume ($p<0.05$) and a strong correlation between BMP-2 and BMP-7 ($p<0.01$).

Conclusion: The results of this pilot study didn't show any significant difference in PICF levels of BMP-2, BMP-7, sRANKL, and OPG in terms of anatomic location of dental implants. Further well-designed studies should be carried out to evaluate the relationship between bone related biomarkers and anatomic location of dental implants.

KEYWORDS

Bone morphogenetic proteins, dental implant, osteoprotegerin, receptor activator of nuclear factor-kappa B

ÖZ

Dental implantların anatomik lokasyonlarının (mandibula ile maxilla) osseointegrasyon süresince peri-implant oluğu sıvısındaki BMP-2, BMP-7, sRANKL ve OPG seviyeleri üzerine etkisi. Pilot bir çalışma

Amaç: Bu çalışmanın amacı, hem maksilla hemde mandibulaya yerleştirilen implantların periimplant oluğu sıvısında osseointegrasyon süresi boyunca kemik morfogenetik protein-2 (BMP-2), BMP-7, çözünür reseptör aktivatör nükleer faktör kappa B ligandı (sRANKL) ve osteoprotegrin (OPG) seviyelerinin araştırılmasıdır.

Gereç ve Yöntemler: Bu çalışmada 33 hasta (17 bayan ve 16 erkek; ortalama yaş 47.03±11.23) yer almıştır. Hem maxillaya (Grup 1/n=18) hemde mandibulaya (Grup 2/n=15) olmak üzere toplam 33 implant yerleştirilmiştir. Peri-implant oluğu sıvısı (PIS) örnekleri, modifiye plak indeksi (MPI), gingival indeks (GI) ve sondlama cep derinliği (SCD) ölçümleri cerrahiden sonra 1. ve 3. ayda alındı. BMP-2/-7, sRANKL ve OPG PIS seviyeleri ELIZA ile incelendi.

Bulgular: İyileşme süresince herhangi bir komplikasyon gözlenmedi.Gruplar arasında sRANKL, OPG, BMP-2 ve BMP-7 PIS seviyeleri ve incelenen klinik parametreler açısından herhangi bir zaman periyodunda anlamlı fark gözlenmedi ($p>0.05$). PIS hacmi 1. ayda grup 2 de grup 1'e göre fazla iken, 3. ayda PIS hacmi grup 1 de grup 2'ye göre fazlaydı ($p<0.05$). PIS hacmi ile sRANKL arasında pozitif ($p<0.05$) ve BMP-2 ile BMP-7 arasında güçlü pozitif korelasyon ($p<0.01$) mevcuttu.

Sonuç: Bu pilot çalışmanın sonuçları, dental implantların anatomik lokasyonları açısından BMP-2, BMP-7, sRANKL, ve OPG PIS seviyelerinde anlamlı bir fark gösterememiştir. Kemik ile ilişkili biyobelirteçler ve dental implantların anatomik lokasyonu arasındaki ilişkinin değerlendirilmesi için iyi dizayn edilmiş çalışmalar yapılmalıdır.

ANAHTAR KELİMELERK

Kemik morfogenetik proteinleri, dental implant, osteoprotegrin, reseptör aktivatör nükleer kappa B ligand

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Osseointegration term has been used to describe a direct structural and functional relationship between living bone tissue and a load-carrying implant surface.[1] A series of some cellular and extracellular biological events occur between bone tissue and implant surface during bone healing around implants.[2] At the bone-implant interface, the expression of several growth and differentiation factors by the activated blood cells, mediates this cascade of biological events.[3] Platelet derived growth factor, insulin-like growth factors, transforming growth factors and fibroblast growth factor enhance bone healing by inducing migration, proliferation, and differentiation into bone cells of mesenchymal-derived cells.[4] Other important bone biological factors that play essential roles in osteogenesis are bone morphogenetic proteins (BMPs). BMPs that belong to the transforming growth factor-β superfamily are secreted signaling proteins which serve as regulating matrix synthesis, cell proliferation and tissue differentiation.[5,6] BMPs stimulate bone tissue formation by differentiating mesenchymal stem cells to osteoblastic and chondroblastic cells.[7] BMP-2 and BMP-7 are the most effective types that induce complete morphogenesis of bone tissue.[8] Like other bone morphogenetic proteins, BMP-2 has an essential function in the development of cartilage and bone tissue.[9] BMP-7 is also known as osteogenic protein-1and plays a key role in the transformation of MSCs into bone and cartilage.[9]

RANKL and OPG are critical factors in the control of osseous healing, which are both produced by osteoblasts. RANKL is a cell membrane-bound protein responsible for stimulation of osteoclast differentiation and bone resorption.[10] OPG (osteoclastogenesis inhibitory factor) counteracts the biological activities of RANKL by preventing its interaction with its receptor (RANK).[11,12] It has been indicated that a balanced RANKL/OPG expression is important in physiological bone remodeling.[13]

The availability of an adequate quantity and quality of bone at the implant site are critical local factors for osseointegration. The quality of bone support differs due to the anatomic location, thus implant outcomes are sometimes categorized according to anatomic location. The types of bones related tothe quality of cortical bone and trabecular bone density were classified by Lekholm and Zarb. According to their classification, the bones types are classified as D1, D2, D3 and D4 bone.[14] The D1 bone consists of dense cortical and trabecular bone.[15] The percentages of light microscopic bone-implant contact (BIC) are highest in D1 bone and more than 80%. Fewer blood vessels are present in D1 bone than in the other three types. The structure of this bone type is almost all cortical and the regeneration capacity is impaired because of the poor blood circulation. D1 bone existsmore often in anterior regions of mandibles. The D2 is composed of a thick crestal layer of dense-to-porous cortical bone and coarse trabecular bone under the cortical bone.[16] In D2 bone there is high amount of blood in contact with the implant surface and primary stability is good.[15] The D2 bone trabeculae are 40% to 60% stronger than D3 trabeculae. This bone is found generally in the anterior region of mandible, followed by the posterior region of mandible. D2 bone type ensures desirable implant healing, and osseointegration is very foreseeable. D3 is composed combination of thinner porous cortical bone on the crest and fine trabecular bone on the inside. The trabecula in D3 bone is approximately 50% more fragile than in D2 bone. D3 bone is found more frequently in the anterior regions of maxilla and posterior regions of both arch. The BIC is also less favorable in D3 bone compared to D2 bone. D4 bone has very few density and small amount or no cortical crestal bone. This type of bone is observed most often in the posterior maxilla. Bone trabeculae are dispersed and, as a result, achievement of primary stability of any implant typepresents a surgical challenge.[16]

Biological factors play an important role in osseointegration, and a few data is present about the biological factors that play essential roles in bone tissue healing and remodeling around dental implants. The purposes of this study were to measure BMP-2, BMP-7, sRANKL, and OPG levels in periimplant crevicular fluid (PICF) around non-submerged implants, which were placed at different locations of maxilla and mandible at 1 and 3 months after surgery; and to correlate these values with clinical parameters.

MATERIALS AND METHODS

Thirty-three patients (17 females and 16 males; mean age 47.03±11.23 years) who were attending the Oral and Maxillofacial Surgery Department at Selcuk University Faculty of Dentistry (during the 2011-2013 academic years) and scheduled for implant surgery were included in this study. The study protocol was approved by the Ethics Commission of Selcuk University Faculty of Medicine for human subjects. None of the patients had a history of systemic disease and had received antibiotics within the prior 6 months. All patients were nonsmokers. Informed consent was obtained from each patient before clinical examination and PICF sampling. All patients received oral hygiene instructions and supragingival scaling. Full-mouth subgingival scaling and root planing under local anesthesia was performed in a single appointment for chronic

periodontitis patients. There was no patient who has ≥4mm periodontal pocket depth after periodontal therapy. A total of 33 implants from single implant system (Nucleoss, Izmir, Turkey) were placed using standard surgical procedures.

Implants were made out of grade 4 titanium in our study. These implants have two-start V-shaped thread pattern and root form body shape. Thread pitch is 0.75 mm and thread depth is 0.3 mm. Bone level implants were used in this study. These implants exhibited 'Maxicell' surface technology, in which the surface is first sandblasted by large grain Al_2O^3, as in the SLA surface, and then is thermal acid etched for surface cleaning. This produces fine 1- to 2-μm micropits on the rough-blasted surface. Average roughness value of the implants (Ra) is 3,2361±0,2315, the root mean square of the values of all points of the surface (Rq) is 4,1316±0,3085, maximum peak-to-valley height of the entire measurement trace (Rt) is 27,1536±3,1756, arithmetic average of the maximum peak-to-valley height of the five greatest values (Rz) is 21,9079±1,6022.17

Implant therapy

Patients who were scheduled for single tooth implant placement surgery in Department of Oral and Maxillofacial Surgery were treated by an experienced surgeon (DD). The implants were kindly provided from the Nucleoss Company. Implants were placed in both of maxilla (Group 1/n=18) and mandible (group 2/n=15) (Table 1). Dental implants were placed using a one-stage protocol. After the administration of local anesthesia (Ultracain DS, Aventis Pharmacheuticals, Istanbul, Turkey), an incision was made on the alveolar ridge. Fifteen implants were placed in the mandible and 18 implants were placed in maxilla. Bone densities at implant sites were judged by the surgeon (DD) during drilling prior to implant placement.[14] Implant sites at mandible exhibited Class II bone density (D2) and implant sites at maxilla exhibited Class III bone density (D3). Good primary stability was obtained for each implant. The mucoperiosteal flaps were adapted around the implant neck to allow non-submerged healing and were sutured with silk sutures (Sterisilk, SSM Sterile Health Products Inc, Istanbul, Turkey). A healing cap (gingival former) was placed for all implants. Postoperative medication included amoxicillin 500 mg 3 times a day for 10 days, paracetamol 500 mg twice a day for 5 days, and 0.2% chlorhexidine mouthwash (Corsodyl, GlaxoSmithKline Consumer Healthcare, UK) twice a day for 10 days. The silk sutures were removed 10 days after surgery. The implants had no probe for resonance frequency analysis. The primary stability was evaluated clinically and by using periapical radiography.

Table 1.

The initial and post-treatment microhardness values (mean±standard

	SEX		Mean Age	Implant Site Distribution		
	Male	Female		Anterior	Premolar	Molar
Group 1	9	9	47.44±11.95	5	11	2
Group 2	7	8	46.53±10.70	0	5	10
Total	16	17	47.03±11.23	5	16	12

Evaluation of the implant status by clinical parameters

The clinical examination included the assessment of pocket depth (PD) and the measurement of the Modified Plaque Index (MPI)[18] and the Gingival Index (GI)[19] MPI and GI measurements were carried out at four sites around each dental implant and PD was performed at six sites around each implant using a periodontal probe with plastic tip. PICF sampling and clinical measurements were recorded at 1 and 3 months after implant therapy.

Collection of PICF and ELISA analysis

PICF was collected via the intracrevicular method.[20] Implant surfaces were dried with air and isolated by cotton rolls. Paper strips (Periopaper, ProFlow, Inc., Amityville, NY, USA) were placed into the crevices of the implants for 30 seconds. The adsorbed volume was established by impedance measurements (Periotron 8000, Oraflow, Inc., Plainview, NY, USA). Two strips (mesial and distal sites) were pooled for each implant. Paper strips were placed into 1.5-mL plastic tubes containing 500 μL of phosphate-buffered saline and stored at −80°C prior to ELISA analysis.

PICF samples were analyzed for sRANKL, OPG (BioVendor, Brno, Czech Republic), BMP-2 and BMP-7(Quantikine, R&D Systems, Minneapolis, MN, USA) using commercially available kits in accordance with the manufacturer's instructions as in our previous study.[21] The absorbance values (optical densities) were measured spectrophotometrically at a wavelength of 450 nm, and the samples were compared with the standards. Data were then calculated and obtained by methods of interpolation of a predetermined standard curve.

Statistical analysis

The statistical analysis was performed using commercially available software (SPSS v.20.0, IBM, Chicago, IL,USA). The Shapiro-Wilks normality test was used to check the normality of the data. Parametric tests were used for statistical analyses.

Independent sample T-test was used in comparison between groups for each time point. Paired t-test was used in comparison between the two time points for the same group. Associations among mean levels of the biomarkers and clinical parameters were also examined using the Spearman rank correlation test.

RESULTS

A total of 33 implants were placed using one-stage protocol. The patient demographic data and implant site distribution were presented in Table 1. The implants showed no clinical signs of peri-implant infection or noticeable mobility during the healing period.

Clinical assessments

PD values (mean ± SD) are reported in Figure 1. There were no significant differences between groups at any time point ($p>0.05$). Statistically significant differences were found in groups between 1and 3 months ($p<0.05$).

MPI values (mean ± SD) are reported in Figure 1. There were no significant differences between groups at any time point and in groups between 1and 3 months ($p>0.05$).

GI values (mean ± SD) are reported in Figure 1. There were no significant differences between groups at any time point and in groups between 1and 3 months ($p>0.05$).

PICF volumes (mean ± SD) are reported in Figure 1. While PICF volume of group 2 was greater than group 1 at first month, PICF volume of group 1 was greater than group 2 at 3 months ($p<0.05$). There was a significant decrease in PICF volume for group 2 at 3 months compared to first month ($p<0.05$).

Biochemical assessments

Total amount (pg/30 sec)

The results of the total amounts of biochemical markers (mean ± SD) between 1 and 3 months are displayed in Figure 2. There were no significant differences between groups at any time point in terms of evaluated biochemical markers ($p>0.05$). No significant differences were found in groups between 1 and 3 months in terms of BMP-2, BMP-7 and sRANKL levels ($p>0.05$). Statistically significant difference was found in group1 between 1and 3 months in terms of OPG levels ($p<0.05$) but not for group 2 ($p>0.05$).

There was no correlation between total amounts of biochemical markers and clinical parameters ($p>0.05$). There was strong positive correlation between sRANKL levels and PICF volume ($p<0.05$). There was a strong positive correlation between BMP-2 and BMP-7 ($p<0.05$).

Concentration (pg/µl)

BMP-2 levels (mean ± SD) are reported in Figure 3. There were significant differences between groups at 1 and 3 months ($p<0.05$). There was a significant decrease in BMP-2 levels for group 1 at 3 months compared to 1 month ($p<0.05$). However, there was a significant increase in BMP-2 levels for group 2 at 3 months compared to 1 month ($p<0.05$).

BMP-7 levels (mean ± SD) are reported in Figure 3. There was significant difference between groups only at 1 month ($p<0.05$). No statistically significant difference was found in group 1 between 1and 3 months in terms of BMP-7 level ($p>0.05$). There was a significant increase in BMP-7 levels for group 2 at 3 months compared to 1 month ($p<0.05$).

sRANKL levels (mean ± SD) are reported in Figure 3. There were significant differences between groups at 1 and 3 months ($p<0.05$). No statistically significant difference was found in group 1 between 1and 3 months in terms of sRANKL level ($p>0.05$). There was a significant increase in sRANKL levels for group 2 at 3 months compared to 1 month ($p<0.05$).

OPG levels (mean ± SD) are reported in Figure 3. There were significant differences between groups at 1 and 3 months ($p<0.05$). There was a significant decrease in OPG levels for group 1 at 3 months compared to 1 month ($p<0.05$). However, there was a significant increase in OPG levels for group 2 at 3 months compared to 1 month ($p<0.05$).

There was no correlation between concentrations of BMP-2, BMP-7, OPG and clinical parameters ($p>0.05$). There was strong negative correlation between sRANKL levels and GI ($p<0.05$). There was a very strong positive correlation between each biochemical parameter ($p<0.001$). There was a very strong negative correlation between each biochemical parameter and PICF volume ($p<0.001$).

We also compared the all implants in terms of specific sites (anterior, premolar and molar regions). We could compare only the implants placed in premolar region statistically. But there was no significant difference in clinical and total PICF levels of evaluated biomarkers.

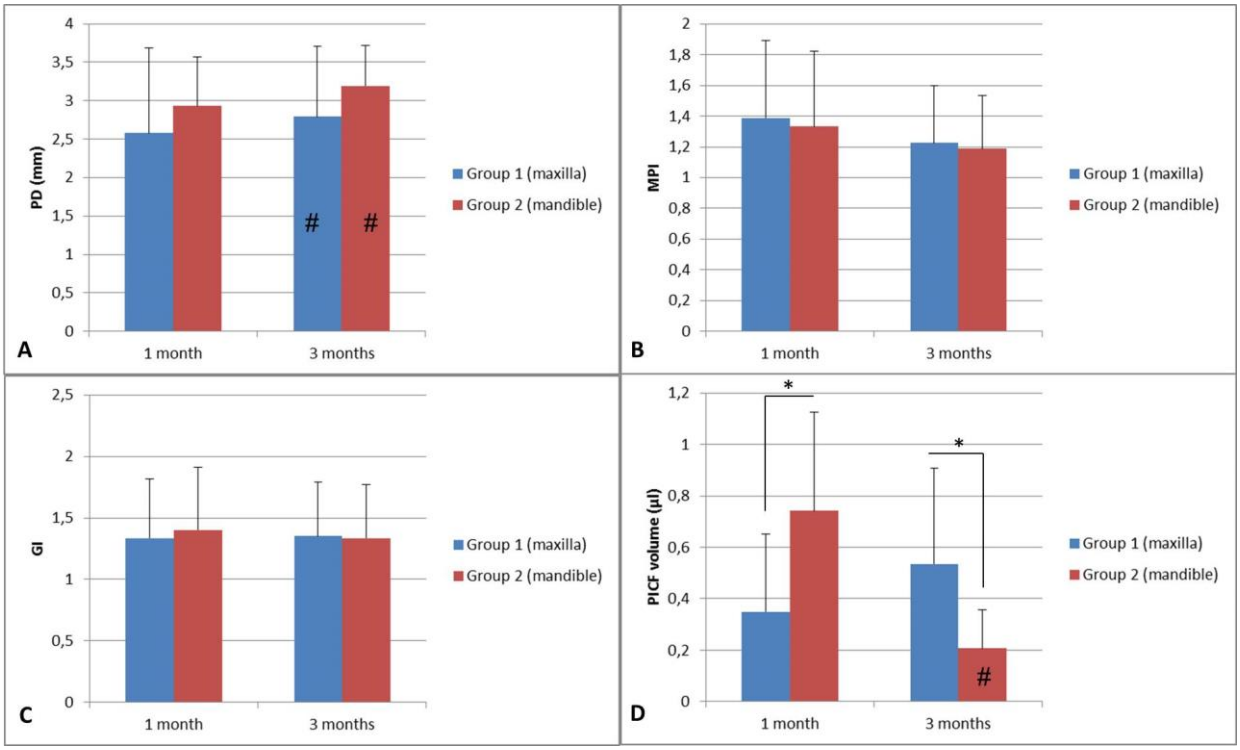

Figure 1.

A) PD (mm) values for each group at 1 and 3 months **B)** MPI values for each group at 1 and 3 months **C)** GI values for each group at 1 and 3 months **D)** PICF volumes (µl) for each group at 1 and 3 months
(*) Significant difference between groups ($p<0.05$). (#) Significant difference compared to the first month

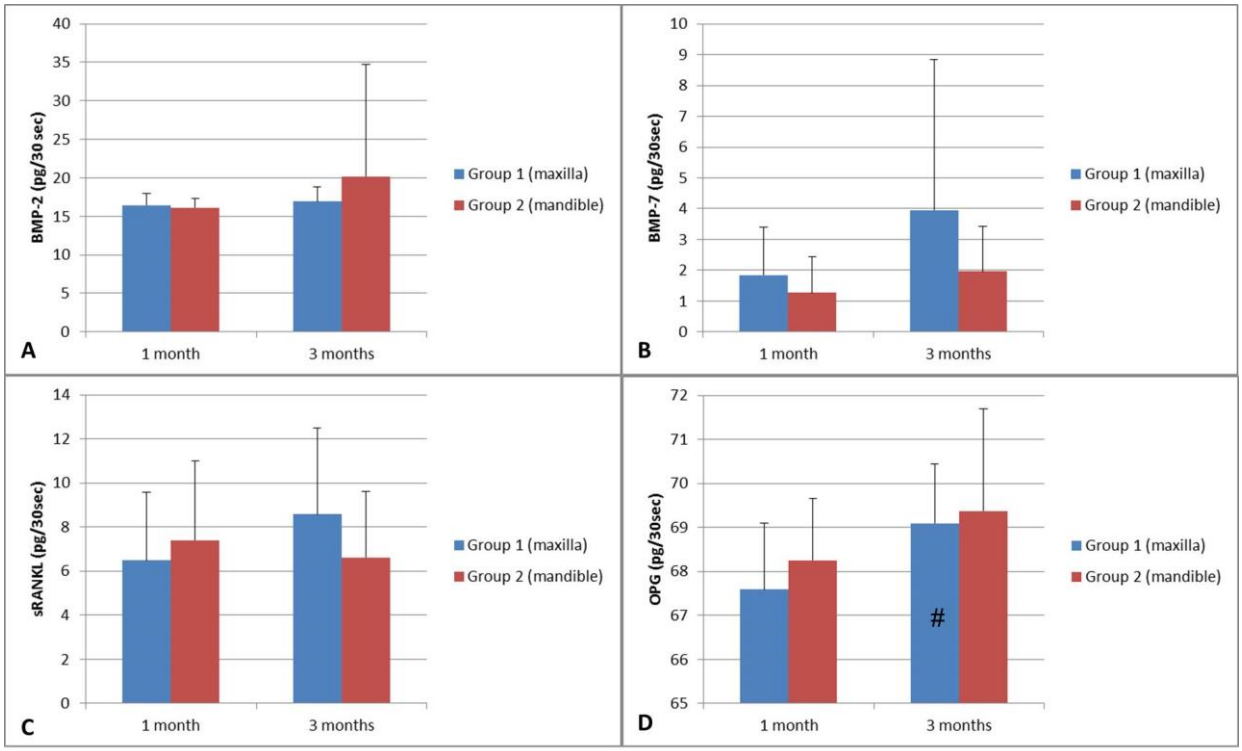

Figure 2.

A) Total amount of BMP-2 in PICF samples for each group at 1 and 3 months **B)** Total amount of BMP-7 in PICF samples for each group at 1 and 3 months **C)** Total amount of sRANKL in PICF samples for each group at 1 and 3 months **D)** Total amount of OPG in PICF samples for each group at 1 and 3 months
(#) Significant difference compared to the first month

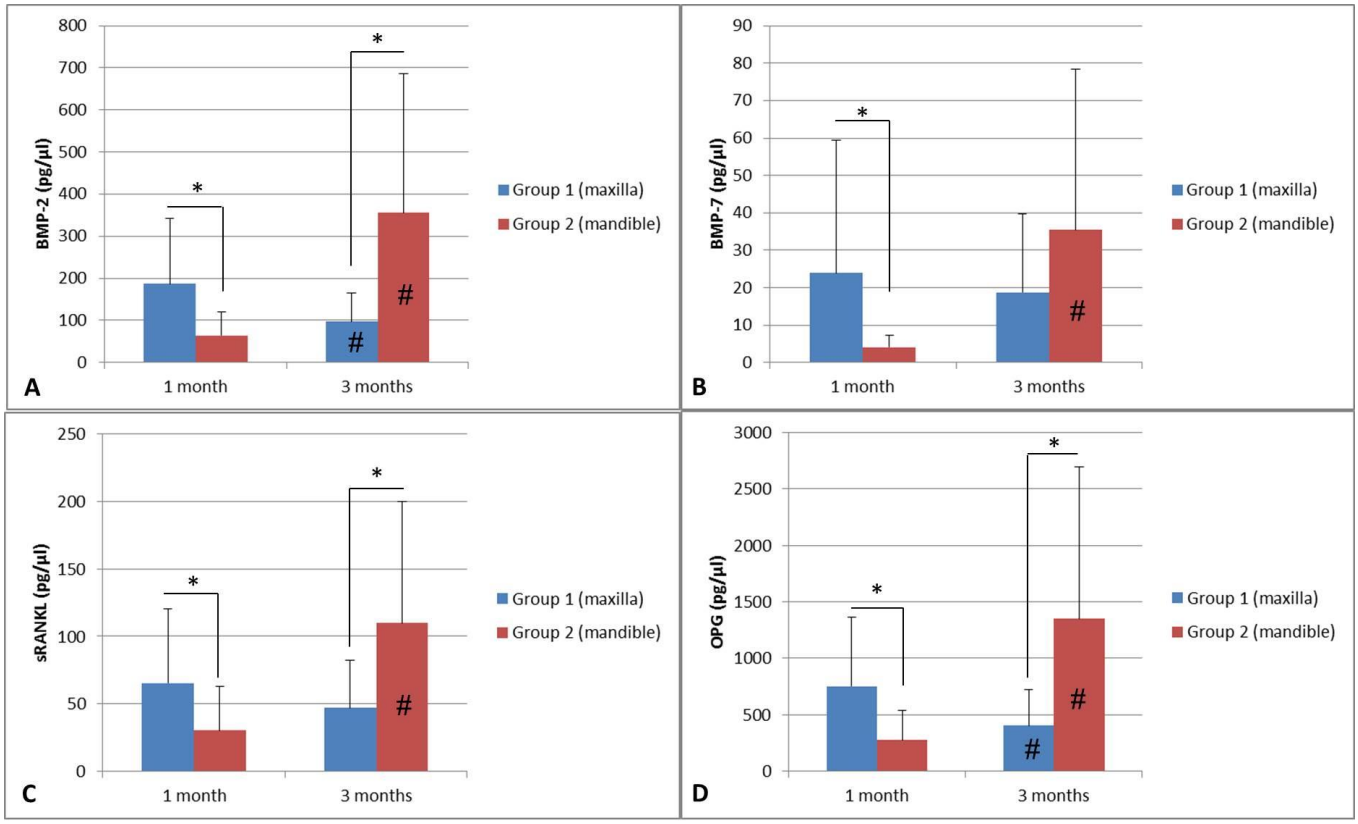

Figure 3.

A) Concentration of BMP-2 in PICF samples for each group at 1 and 3 months **B)** Concentration of BMP-7 in PICF samples for each group at 1 and 3 months **C)** Concentration of sRANKL in PICF samples for each group at 1 and 3 months **D)** Concentration of OPG in PICF samples for each group at 1 and 3 months

(*) Significant difference between groups ($p<0.05$). (#) Significant difference compared to the first month

DISCUSSION

Clinical performance of dental implants may be related to the bone quality of different anatomical regions. It has been indicated that success rate of implants in the mandible seems to be lightly greater than in maxilla with a 4% difference. The success percentage of implants in the anterior regions seems to be greater than in the posterior regions, particularly owing to the quality of bone: about 12% difference between anterior and posterior regions of maxilla, and about 4% difference between anterior and posterior regions of mandible.[22] Trabecular bone has a very high surface area, which is contiguous with the marrow fragment. Since marrow contains not only mesenchymal progenitor cells that have differentiation capability into osteoblasts, but also a rich vascularity that can supply both the circulating mononuclear precursors to osteoclasts (needed for remodeling) and the endothelial population needed for angiogenesis, it is not surprising that trabecular bone can remodel far more quickly than cortical bone.[23] D2 bone has dense to thick porous cortical bone on the crest and coarse trabecular bone underneath. The bone density D2 is the most common bone density observed in the mandible. D3 bone has a thinner porous cortical crest and fine trabecular bone within. Bone density D3 is very common in maxilla. The strength of D3 bone is 50 %weaker than D2 bone. D2 bone usually has 65% and 75% BIC after initial healing; D3 bone typically has 40% and 50% BIC after initial healing.[24]

In this present study, we compared the implants placed in mandible (D2) and maxilla (D3 bone) in terms of the levels of BMP-2, BMP-7, sRANKL, and OPG in PICF. It has been suggested that total amounts of cytokines in the GCF sample rather than concentration is a better marker of relative GCF ingredient activity because concentrations are directly affected by the volume of the sample.[25,26] Thus, we based our discussion was based mainly on the total amount of data, despite both total amounts and concentrations were measured and presented.

BMPs, particularly BMP-2 and BMP-7 are able to increase notably all the cellular events during osteoblastogenesis, such as proliferation, differentiation, mineralization and migration.[27] In our previous study, we have compared Straumann

SLActive and SLA surface implants with Nucleoss implants used in this study in terms of PICF levels of BMP-2, BMP-7, sRANKL and OPG. All implants were placed in mandible only and we observed that PICF levels of BMP-2 and BMP-7 were similar between all implant systems at 1 and 3 months.[21] In this present study, there was also no significant difference in PICF levels of BMP-2 and BMP-7 between groups at all-time points. Vlacic-Zischike et al. indicated that a number of genes related tothe TGFβ-BMP signaling cascade (BMP2, BMP6, CREBBP, SP1, RBL2, ACVR1, TBS3, and ZFYVE16) were significantly differentially upregulated with culture on the SLActive surface and BMP2 expression has the largest fold change increase, which was consequently affirmed at the protein level by ELISA.[28] Mamalis et al. reported that after 7 days of culture, the gene expression of BMP-7 by hPDL cells was significantly upregulated in response to the SLActive surface compared to the SLA surface[29] Eriksson et al. reported that more BMP-2-positive cells were observed on hydrophilic titanium discs than on hydrophobic ones after 1 week.[30] It was demonstrated that surface roughness induced BMP-2 mRNA expression, especially at the early time point of 24 h.[31] According to these studies, we think that differences in BMP-2 and BMP-7 levels may be due to implant surface topography, especially in early time periods. In present study, only one type implants (SLA) were used and it is not an unexpected event that we couldn't find any difference in terms of BMP-2 and BMP-7 levels in PICF. We observed a strong positive correlation between BMP-2 and BMP-7 levels in PICF.

RANKL/RANK/OPG system is essential for bone homeostasis. The binding of RANKL to its receptor RANK on the surface of pre-osteoclasts induces their differentiation into mature osteoclasts, thus leading to bone resorption.10 It is expressed by activated T and B cells, osteoblasts, periodontal, gingival fibroblasts and epithelial cells.[32-34] Osteoprotegerin (OPG) is a soluble tumor necrosis factor receptor-like molecule that serves as a decoy receptor and blocks the binding of RANKL to RANK and thus inhibits osteoclastogenesis. OPG is expressed by osteoblasts, periodontal ligament cells, gingival fibroblasts and epithelial cells.[32,35] In our previous study, we also observed that PICF levels of sRANKL and OPG were similar between all implant systems at 1 and 3 months.[21] In this present study, there was no significant difference in PICF levels of sRANKL and OPG between groups at all-time points. In two studies researched sRANKL levels in PICF, no significant correlation was found between the PICF levels of sRANKL and the clinical parameters (PD, MPI and GI) measured around the dental implants.[36,37] We couldn't find any significant correlation between the PICF levels of sRANKL and the clinical parameters as in these studies. Monov et al[36] detected sRANKL in 35% of their samples, whereas Arikan et al[37] detected sRANKL in 12% of the samples in their studies. We detected sRANKL in all of the samples as in the study of Sarlati et al.[38] Our findings can be explained by the periodontal health status of the dental implants. Such a difference may account for different levels of sub-clinical inflammation among healthy subjects or differences in the sensitivity of various ELISA kits employed in each study.[39] There was strong positive correlation between sRANKL levels and PICF volume in this present study. The detected sRANKL levels may be associated with sub-clinical inflammation or bone remodeling in the dental implants in our study. Güncü et al. reported that although the PICF RANKL level in gingivitis/inflamed group was higher than the level of healthy/non-inflamed group, the difference between groups did not reach the statistically significant level.[40] Sarlati et al. also demonstrated that there were no statistically significant differences in sRANKL concentration between healthy group, peri-implant mucositis and periimplantitis.[38] In our study, there was no clinical inflammation signs (edema, bleeding, change in color or pus formation) in the placed implants, thus we think that finding a difference in RANKL levels of healthy implants is difficult considering the findings of studies mentioned above. It was indicated that levels of RANKL, OPG, M- CSF and other mediators involved in osteoclast formation are also regulated in response to different metal particles in vitro and these differences may reflect the osteoclastogenic potential of different chemical composition of biomaterials.[38]

Rausch-fan et al. reported that OPG production of primary human alveolar osteoblasts and human osteoblast-like MG63 cells were higher in SLActive surfaces compared to SLA surfaces in in vitro conditions.[41] In another study, it was demonstrated that the gene expression of OPG by hPDL cells, which have osteoblast-like properties, was significantly upregulated in response to the SLActive surface compared to SLA surface.[29] According to the findings of these studies, we think that differences in PICF OPG levels may be due to implant surface topography as PICF BMP-2 and BMP-7 levels in our present study. Güncü et al. demonstrated that PICF OPG levels were significantly greater in gingivitis/inflamed group compared to healthy/non-inflamed group by using ELISA.[40] However, Hall et al. demonstrated that OPG levels in PICF were similar for the subjects in the healthy and peri-implantitis group by using quantitative polymerase chain reaction.[42] Differences in these studies may be due to analyze method, PICF sampling procedure, study population. We didn't observe any differences in OPG level between groups. This condition may be related to clinically healthy condition of all implants.

Arikan et al. showed that the total amount of OPG was positively correlated with gingival index, BOP, and PICF volume.[37] They suggested that locally produced OPG correlated with the local signs of inflammation in periodontal and/or peri-implant tissues. However, we observed that no significant correlation between OPG, PICF volume and other evaluated clinical parameters in this present study. The population of a study by Arikan et al. had an unbalanced distribution of samples into three periodontal health categories.[37] They investigated 79 healthy implants, four implants with peri-implant mucositis and three implants with peri-implantitis. In our study, all implants were healthy. The differences in correlations between their study and ours may be due to this situation.

There were no significant differences in clinical parameters between groups at 1 and 3 months. PD values significantly increased at 3 months compared to first month in both groups. Some authors concluded that increased pocket depth could be correlated with a higher degree of inflammation of the peri-implant mucosa.[43-45] In our study, there was a strong positive correlation between PD and GI values but there wasn't any increase in GI values at 3 months compared to first month in both groups. All implants were already clinically healthy. Bengazi et al reported that a slight decrease in mean probing depth (0.2 mm) in Brånemark oral implants with fixed prosthesis at follow up period and apical migration of the soft tissue margin mainly occurred during the first 6 months of observation period. They suggested that the recession of the periimplant soft tissue margin mainly may be the result of a remodelling of the soft tissue.[46] Other investigators reported that a significant increase in peri-implant probing values.[47,48] But follow up periods were 1 and 3 years in these studies. It was also concluded that peri-implant PD measurements are more sensitive to force variation than the corresponding measurements around teeth.[49] This situation also may be the reason of increased PD values in this study although clinical parameters were measured by the same examiner.

PICF volume values were significantly different between groups at all-time points. There was no correlation between PICF volume and clinical parameters. While PICF volume change was not significant for group 1, PICF volume decreased at 3 months compared to first month for group 2. Although all implants were clinically healthy, the changes in PICF volume may be related to sub-clinical inflammation around dental implants.

In our study, first and 3 months after surgery were chosen for the PICF sampling times. We waited for complete epithelial healing to prevent the possible effect of inflammatory events on biomarkers in PICF. A fully epithelialized gingival crevice with a well-defined epithelial attachment can be occurred one month after flap surgery. Furthermore, woven bone is the first bone tissue that is formed in osseointegration and its formation clearly dominates the healing area within the first 4 to 6 weeks after surgery.[50] Thus, the first month after surgery was decided to be the first time point for PICF sampling. A healing time of 3 to 6 months was recommended for the conventional protocol of implant loading.[51] In our study, implant loading was performed at 3 months after surgery and this time period was decided to be the second PICF sampling time. Prosthetic appointments were arranged for the patients after completing PICF samplings.

CONCLUSION

In our pilot study, the significant effect of anatomic location on the levels of BMP-2, BMP-7, sRANKL, and OPG in PICF was not observed. Both volume and density of available bone are important factors for osseointegration of dental implants. Bone volume and density varies from site to site and from patient to patient. In our study, implants in mandible were placed in D2 bone and implants in maxilla were placed in D3. There were no implants in D1 and D4 bones. The lack of these groups was a limitation in our study. This was a pilot study and it was not possible to calculate a power analysis to determine the number of implants in each group. In view of our findings, further well-designed studies with sample size needed for ≥ 80% statistical power could be conducted, and different time points (might be earlier) and other biochemical markers might be chosen for PICF sampling to evaluate the relationship between anatomic location of implants and bone remodeling parameters.

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