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TATIANA ONUMA

NÍVEIS DO LIGANTE RECEPTOR ATIVADOR DO FATOR NUCLEAR κβ (RANKL) E OSTEOPROTEGERINA (OPG) NO FLUIDO CREVICULAR PERI-IMPLANTAR DE IMPLANTES CARREGADOS IMEDIATAMENTE EM PACIENTES COM OSTEOPENIA

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Co-orientadora: Profa*.* Dra*.* Poliana Mendes Duarte

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Guarulhos, 25 de Fevereiro de 2011.

DEDICATÓRIA

A minha família:
Meu pai Gaspar, obrigado por me ajudar
a subir mais um degrau. Devido ao seu estímulo,
hoje sou o que sou graças a você.
A minha mãe Kikue, que sempre esteve do meu lado.
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RESUMO

O sucesso dos implantes carregados imediatamente é influenciado por uma série de fatores de confundimento, como a resposta da remodelação óssea e qualidade óssea na região peri-implantar. A reabsorção óssea é controlada pela interação do ligante do receptor ativador do fator nuclear κß (RANKL) e osteoprotegerina (OPG). RANKL induz a formação e ativação dos osteoclastos por se ligar com o receptor ativador do fator nuclear κß (RANK), enquanto OPG é um receptor para RANKL que inibe a osteoclastogênese. O objetivo deste estudo prospectivo controlado foi avaliar os níveis dos fatores relacionados com a osteoclastogênese (RANKL e OPG) no fluido crevicular peri-implantar (FCPI) de implantes imediatamente carregados em indivíduos com e sem osteopenia após 120 dias do carregamento. Vinte e três pacientes foram divididos de acordo com critérios estabelecidos pela Organização Mundial da Saúde: controle (n = 10 pacientes; T-score $\geq$ -1) e osteopenia (n = 13 pacientes; -1 <T-score $\leq$ -2,5). Os parâmetros clínicos e imunológicos foram coletados no tempo 7 e 120 dias após a cirurgia. Oitenta e oito implantes foram carregados imediatamente sendo 38 implantes no grupo controle e 50 no grupo de osteopenia. Os níveis de RANKL, OPG e a proporção RANKL:OPG bem como os parâmetros clínicos foram semelhantes entre os grupos em ambos os períodos (p> 0,05), apesar de existir diferenças significativas entre o tempo 7 e 120 dias de pós-cirurgia e níveis de FCPI (p <0,001). Dentro dos limites deste estudo, pode ser sugerido que a osteopenia não influenciou a resposta do tecido peri-implantar ao redor de implantes carregados imediatamente, após 120 dias de pós-cirurgia.

Palavras-Chaves: Implante Dental, Carga Imediata, Osteopenia, Osteoporose, RANKL, OPG.

ABSTRACT

The successful outcome of immediately loaded implants is influenced by a number of confounding factors, such as bone remodeling response and bone quality in the peri-implant site. Bone resorption is controlled by the interaction of the receptor activator of the NF-κß ligand (RANKL) and osteoprotegerin (OPG). RANKL induces osteoclast formation and activation for binding on the receptor activator of the NF-κß (RANK), while OPG is a decoy receptor for RANKL that inhibits osteoclastogenesis. The aim of this prospective-controlled study was to evaluate the osteoclastogenesis-related factors (RANKL and OPG) levels in the peri-implant crevicular fluid (PICF) of immediately loaded implants in patients with and without osteopenia after 120 days of loading**.** Twenty-three patients were divided according to criteria established by the World Health Organization: control (n=10 patients; T-score $\geq$ -1) and osteopenia (n=13 patients; -1< T-score $\leq$ -2.5). Clinical parameters and PICF were taken at baseline and 120 days after surgery. Eighty-eight implants were immediately loaded being 38 implants in control group and 50 in the osteopenia group. The levels of RANKL, OPG and RANKL:OPG ratio as well as clinical parameters were similar between groups in both periods ($p>0.05$), although there were significant differences between baseline and 120 days post-surgery PICF levels ($p<0.001$). Within the limits of this study, it could be suggested that osteopenia did not influenced the peri-implant tissue response around immediately loaded implants, after 120 days post-surgery.

Key-Words: Dental Implants, Immediate loading, Osteopenia, Osteoporosis, RANKL, OPG.

SUMÁRIO

1. INTRODUÇÃO E JUSTIFICATIVA

A reabilitação oral protética dos pacientes portadores de edentulismo total ou parcial através dos implantes osseointegrados tem sido uma alternativa amplamente empregada e muito bem sucedida (Astrand et al., 2008; Chiapasco et al., 2011; Friberg et al., 2001; van Steenberghe et al., 1999).

A técnica convencional de instalação de implantes osseointegrados preconiza um período de cicatrização ou osseointegração que varia de de 2 a 4 meses para implantes de superfícies tratadas. Entretanto, a técnica de utilização de implantes ativados imediatamente tem apresentado resultados muito promissores (Degidi et al., 2006; Degidi et al., 2008_b, Glauser et al., 2007; Nkenke e Fenner, 2006; Sadowsky, 2011), com índices de sucesso, que variam entre 85 a 100%, tanto para restaurações quanto para o carregamento imediato (den Hartog et al., 2011; Glauser et al., 2007; Nkenke e Fenner, 2006). Ativação imediata foi definida como à inserção de uma estrutura protética ou componente protético associado a uma restauração implanto-suportada provisória em até 48 horas após a cirurgia de inserção do implante (Cochran et al., 2004). Estes procedimentos têm como objetivos principais a redução do número de intervenções cirúrgicas e a diminuição do tempo de tratamento entre as fases cirúrgica e protética, oferecendo ao paciente a mesma previsibilidade do tratamento convencional. As restaurações podem ainda ser classificadas quanto ao tipo de oclusão: carregamento imediato, no qual a prótese provisória implanto-suportada apresenta contato oclusal com o arco antagonista ou restauração imediata, no qual não há contato direto entre a restauração implanto-suportada e o arco antagonista. A restauração imediata é muito empregada nos casos de próteses implanto-suportadas unitárias ou de até três elementos protéticos (Degidi et al., 2008_a e 2009). Recentes revisões

sistemáticas tem mostrado altos índices de sucesso de implantes carregados ou restaurados imediatamente, principalmente na mandíbula (Nkenke e Fenner, 2006).

A alta previsibilidade desta técnica é decorrente do desenvolvimento de novas macro- e microestruturas (Grassi et al., 2006; Nkenke e Fenner, 2006; Shibli et al., 2007 e 2010) além dos altos índices de sucesso já reportados anteriormente. Entretanto, as perdas destas restaurações implanto-suportadas podem comprometer o tratamento reabilitador. As perdas ou falências podem ser classificadas em precoce causadas por fatores locais e sistêmicos do indivíduo (Alsaadi et al., 2008; Esposito et al., 1998; Quirynen et al., 2002; Shibli et al., 2005); e em perdas tardias relacionadas à infecção bacteriana, também conhecidas como peri-implantites (Sakka e Coulthard, 2011; Shibli et al., 2003 e 2006) ou através das sobrecargas oclusais (Esposito et al., 1998; Sakka e Coulthard, 2011).

Os fatores sistêmicos relacionados a perda precoce podem interferir nos eventos celulares básicos referentes à aposição e maturação do tecido ósseo ao redor do implante (van Steenberghe et al., 2002, 2003), resultando na interposição de tecido conjuntivo denso entre o tecido ósseo e a superfície do implante (Esposito et al., 1999). Fatores sistêmicos como idade, doenças imunossupressoras, diabetes, doenças cardiovasculares, os hábitos como o fumo e recentemente a osteoporose parecem influenciar a longevidade das restaurações implanto-suportadas (Alsaadi et al., 2008). Com o crescente aumento da expectativa de vida da população brasileira (HTTP://www.datasus.gov.br acessado em 10/02/2011) e o crescente aumento da utilização de reabilitações implanto-suportadas, a osteoporose vem despertando grande interesse na classe odontológica (Jeffecoat e Chesnut, 1993; Shibli et al. $2008_{a,b,c}$).

A osteoporose é uma doença crônica, multifatorial e sistêmica que diminui a massa óssea e deteriora a microarquitetura do tecido ósseo fazendo com que o

indivíduo venha a ter um maior risco a fratura (Consensus Development Conference on Osteoporosis, 1993). Normalmente está correlacionado com a idade, e é encontrado especialmente após a menopausa em mulheres (Friberg, 1994; Glösel et al., 2010; Jeffcoat, 2006; Mellado-Valero et al., 2010). Apesar desta doença aparentemente estar relacionada com a perda clínica de inserção periodontal, ainda não existem estudos clínicos que apresentem uma correlação direta entre o insucesso do implante osseointegrado e a osteoporose (Elsubeihi et al., 2002; van Steenberghe, 2002). Podem ser encontrados relatos na literatura sobre a contra-indicação dos implantes osseointegrados, ou que o mesmo pode ser considerado como procedimento de risco em pacientes com osteoporose, devido ao fato de que doenças metabólicas podem afetar o tecido ósseo dos arcos dentários, da mesma forma que afetam outras partes do esqueleto, como a coluna lombar e fêmur (Jeffcoat, 2006; Mellado-Valero et al., 2010). Complementarmente, vários estudos tem apontado elevados índices de perdas de implantes osseointegrados em áreas de osso tipo IV (Friberg et al., 1991; Grassi et al., 2006; Jaffin e Berman, 1991; Quirynen et al., 1991; Shibli et al., 2007).

Em indivíduos adultos, o tecido ósseo apresenta uma dinâmica e uma constante remodelação, em resposta ao estresse mecânico e alterações hormonais. Esta remodelação ocorre a partir de unidades esqueléticas denominadas unidades de remodelação óssea ou *bone remodeling units (BMU)* e envolve um equilíbrio dinâmico entre a reabsorção óssea realizado por osteoclastos e a aposição óssea por osteoblastos (Manologas, 2000; Vega et al., 2007). Cada ciclo de remodelação da BMU inicia-se a partir da transformação de uma superfície óssea latente ou inativa para uma superfície óssea reabsorvida, também denominada de lacuna de *Howship* após ativação dos osteoblastos e osteoclastos via sistema canalicular. A reabsorção do tecido ósseo no processo de remodelação termina com a apoptose dos osteoclastos seguida pela

ativação celular dos osteoblastos que sintetizam matriz óssea que será mineralizada extracelularmente, com a deposição da matriz óssea alguns osteoblastos ficam aprisionados, estes osteoblastos recebem o nome de osteócitos.

Embora haja evidências de que a deficiência de estrógeno estimule a reabsorção óssea por meio de citocinas que aumentam a formação de osteoclastos, os fatores que regulam todo o processo ainda não estão totalmente elucidados (Qiu et al., 2006; D´Amelio et al., 2008). A atividade dos osteoclastos é regulada por várias citocinas como as interleucinas-1, -6 e -11 (IL-1, IL-6, IL-11), alguns hormônios como o paratormônio (PTH), o 1,25-dihidroxi vitamina D3 e calcitonina. O fator de necrose tumoral (*tumor necrosis factor – TNF*), principalmente o TNF-α é também um dos reguladores do processo de reabsorção óssea agindo diretamente na estimulação dos precursores osteoclásticos e indiretamente no controle do sistema osteoprotegerina (OPG), ligante do receptor ativador do fator nuclear κß (RANKL), e receptor ativador do fator nuclear κß (RANK), estas, são consideradas moléculas fundamentais no metabolismo ósseo. Resumidamente, a formação (osteoclastogênese) e atividade dos osteoclastos e, consequentemente, a reabsorção do tecido ósseo inicia-se a partir da ligação RANK/RANKL, enquanto que a OPG que é produzida pelos osteoblastos, é capaz de regular este processo de reabsorção devido a sua capacidade de união ao RANKL, evitando assim a interação RANK/RANKL (Tanaka et al., 2005), portanto a OPG agem como engodo que compete com a RANKL (Özmen et al. 2007). Esta interação inibe a proliferação e diferenciação de osteoclastos, prevenindo a reabsorção óssea. Durante a menopausa, o processo de reabsorção e aposição óssea sofre um desequilíbrio, no qual há um estímulo no processo de osteoclasia, por meio do aumento da produção de RANKL e TNF pelos monócitos e células T.

Neste ínterim, vários estudos têm avaliado e quantificado a osseointegração sobre a influência da osteoporose, tanto em estudos experimentais utilizando modelos animais (Duarte et al., 2003 e 2005; Giro et al., 2007 e 2008; Glösel et al., 2010; Ozawa et al., 2002; Sakakura et al., 2006), avaliações clínicas (Amorin et al., 2006; Holahan et al., 2008) e histolológicas em humanos (Melo et al., 2008; Shibli et al., $2008_{a,b,c}$).

Experimentos utilizando modelos animais (Duarte et al., 2003; Giro et al., 2007 e 2008; Glösel et al., 2010; Okamura et al., 2004; Ozawa et al., 2002; Sakakura et al., 2006), têm mostrado que a osteoporose influência o processo de osseointegração, principalmente na porção óssea medular. Estudos clínicos avaliando a longevidade de implantes inseridos em pacientes com osteoropose são quase sempre retrospectivos (Alsaadi et al., 2008; Holahan et al., 2008; van Steembergue et al., 2003) ou utilizam avaliações apresentando resultados pouco conclusivos (Amorin et al., 2006).

Estudo realizado por Holahan et al. 2008 por meio de estudo restrospectivo, avaliaram 3224 implante inseridos em 746 mulheres com 50 anos ou mais, divididas em pacientes do grupo saúde, osteopenia e osteoporose, sendo que o diagnóstico de osteopenia/osteoporose não foram significantes para a perda do implante quando comparado com o grupo que não tinha a doença. Os autores sugeriram que o diagnóstico de osteopenia e osteoporose não contribuiu de forma efetiva para elevar o risco da perda de implante.

Em 2008, Alsaadi et al. realizaram um estudo retrospectivo para avaliar a influência de fatores sistêmicos e locais na ocorrência de perda de implante com dois anos de conexão protética. Para os 412 pacientes que apresentavam 1514 implantes instalados, onde foram analisados as alterações sistêmicas que influenciassem a perda do implante como hipertensão, problemas de coagulação, osteoporose, hipo-

hipertireoidismo, quimioterapia, diabetes e fumo. Como resultado das avaliações, os principais problemas que afetaram a perda do implante foram radioterapias, diâmetro e localização do implante. Não foi encontrada nenhuma correlação entre perda do implante com osteopenia/osteoporose.

Avaliando histologicamente implantes fraturados ou perdidos, removidos de pacientes com e sem osteoporose, Shibli et al. $2008_{a,b,c}$ em uma série de estudos observaram que não há diferenças entre o percentual de contato osso-implante entre estes pacientes, pelo menos após a osseointegração. Embora seja um resultado oriundo de estudo histológico retrospectivo, estes achados levantaram importantes questionamentos sobre a reabilitação de indivíduos osteoporóticos utilizando implantes osseointegrados.

Dentro deste contexto, a osteopenia é uma interface entre o estado normal e a doença osteoporose, sendo a mesma referida com a densidade mineral óssea intermediária entreambos. O diagnóstico de osteopenia pode significar um grande risco para o futuro desenvolvimento da osteoporose (Kanis et al. 1994; WHO 2007).

2. PROPOSIÇÃO

O objetivo geral deste estudo prospectivo, controlado e longitudinal foi avaliar os fatores clínicos e os relacionados com a osteoclatogênese (RANKL e OPG), sobre influência da osteopenia nos implantes osseointegrados de ativação imediata após 120 dias.

3. ARTIGO

Receptor of activator of the NF-Κß ligand (RANKL) and osteoprotegerin (OPG) levels in the peri-implant crevicular fluid of immediately loaded implants in patients with osteopenia: **a short-term report** *(preparado segundo as normas do Clinical Implant Dentistry and Related Research)*

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ABSTRACT:

Background: The successful outcome of immediately loaded implants is influenced by a number of confounding factors, such as bone remodeling response and bone quality in the peri-implant site. Bone resorption is controlled by the interaction of the receptor activator of the NF-κß ligand (RANKL) and osteoprotegerin (OPG). RANKL induces osteoclast formation and activation, while OPG is a decoy receptor for RANKL that inhibits osteoclastogenesis. **Purpose:** This prospective-controlled study evaluated the levels of osteoclastogenesis-related factors (RANKL and OPG) in the peri-implant crevicular fluid (PICF) of immediately loaded implant in patients with and without osteopenia after 120 days of loading**. Methods:** Twenty-three patients were divided according to criteria established by the World Health Organization: control (n=10 patients; T-score ≥ -1) and osteopenia (n=13 patients; -1< T-score ≤ -2.5). Clinical parameters and PICF were taken at baseline and 120 days after surgery. **Results:** 88 implants were immediately loaded being 38 implants in control group and 50 in the osteopenia group. The levels of RANKL, OPG and RANKL:OPG ratio as well as clinical parameters were similar between groups in both periods ($p>0.05$), although there were significant differences of PICF levels between baseline and 120 days post-surgery ($p<0.001$). **Conclusion:** Within the limits of this study, it could be suggested that osteopenia did not influenced the peri-implant tissue response around immediately loaded implants, at least, after 120 days post-surgery.

Key-Words: Dental Implants, Immediate loading, Osteopenia, Osteoporosis, RANKL, OPG.

INTRODUCTION

Primary implant stability and lack of micromovement were considered to be the main factors involved in the success of immediate loaded implants.[1-2] However, the successful outcome of immediately loaded implants is also influenced by a number of confounding factors such as the bone remodeling response, implant design, surface topography and clinical protocols. Bone quality and quantity are another important factors, with a higher failure rate having been observed in implants placed at type IV bone. Bone resorption is controlled by the interaction of the receptor activator of the NF-kB ligand (RANKL) and osteoprotegerin (OPG). RANKL induces osteoclast formation and activation, while OPG is a decoy receptor for RANKL that inhibits osteoclastogenesis.

Osteoporosis is a disease that influences the quality of bone tissue such that it may become susceptible to fracture. Osteopenia is a term to define bone density that is not normal but also not as low as osteoporosis. By definition from the World Health Organization osteopenia is defined by bone densitometry as a T score −1 to −2.5. There are many causes for osteopenia including calcium and vitamin D deficiency and inactivity. Genetics plays an important role in a person's bone mineral density and often-Caucasian women with a thin body habitus who are premenopausal are found to have osteopenia.

Concern over dental implants being contraindicated in patients with osteoporosis and osteopenia is based on the assumption that this metabolic disease affects the jaws in the same way as it affects other parts of the skeleton, such as the lumbar spine, femur, neck and forearm. The mechanism by which osteoporosis/osteopenia acts on peri-implant bone is based on the decrease in both cancellous bone volume and bone-to-implant contact, consequently reducing the bone tissue available to support dental implants.[3]

Osteotropic factors, e.g., parathyroid hormone, Vitamin D3 or prostaglandin, increase the ratio between RANKL and OPG in favor of RANKL and can support osteoclastogenesis, whereas estrogens, for example, can inhibit osteoclast recruitment by changing the RANKL/OPG ratio in favor of OPG.[4]

On the other hand, the strictly regulated interaction of osteoblasts and osteoclasts could be influenced by mechanical load. Several studies showed that mechanical load could modulate the key factors controlling osteoclast recruitment. A previous study[5] demonstrated a mechanically induced down-regulation of RANKL and an up-regulation of endothelial nitric-oxide synthase, an enzyme producing the signaling molecule nitric-oxide, which is supposed to prevent bone resorption. Other study[6] found that mechanical stimulation significantly increased OPG levels and decreased the presence of macrophage-colony stimulating factor without affecting RANKL. Both studies suggested that the observed effects could lead to decreased osteoclast activity and hypothesized that mechanical loading not only stimulates bone formation but also inhibits its resorption. In contrast to these studies, mechanical loading increased RANKL expression during mandibular distraction, and also in human periodontal cells, and in primary murine osteoblasts suggesting a stimulating effect on osteoclast recruitment.[7-9]

Osteoporosis/osteopenia is thought to be a result of an altered bone-remodeling process, i.e. bone-tissue formation decreases while resorptive capacity remains constant. In addition, osteoporosis and osteopenia may represent a contraindication or risk factor for osseointegration, but this is still controversial.

Therefore, the aim of this prospective-controlled study was to evaluate the cytokine levels of the osteoclastogenesis-related factors (RANKL and OPG) in the peri-implant crevicular fluid (PICF) of immediately loaded implants with platform switching in

patients with and without osteopenia in a short term follow-up.

Material and Methods

Selection of the subjects

Twenty-three females with a mean age of 61.6±5.97 years presenting completely or partially edentulous mandible were included in this study. These patients were divided according to criteria established by the World Health Organization: control (*n*=10 patients; T-score ≥ -1) and osteopenia (*n*=13 patients; -1< T-score ≤ -2.5). T-score was based on the bone mineral density (BMD) that was measured at the spine and hip by dual-energy x-ray absorptiometry (DXA) device.

These patients fulfilled the following inclusion criteria: adequate amount of bone height for placement of implants with a minimum length of 10mm in a prosthetically optimal position, implant site free from acute infection or extraction remnants and primary stability ≥30N/cm. Placement of the implant immediately after tooth extraction without regenerative procedures was also accepted in the study design.

Exclusion criteria included local radiation therapy, smoking, absence of primary stability of the implant (<30Ncm), need of local bone regeneration procedures, previous bone augmentation in the implant site, moderate to severe chronic periodontitis (i.e., suppuration, bleeding on probing in more than 30% of the subgingival sites or any site with probing depth ≥5mm), diabetes or any systemic condition that could affect the bone healing. The Ethics Committee for Human Clinical Trials at Guarulhos University approved the study protocol (#147/08), which was explained to each subject, and all patients signed informed consent.

Calculation of the sample size was based on previous study.[10] A difference of 20% in alveolar bone crestal remodeling/bone loss and implant success rate between the implants from different groups (with or without osteopenia) was set. With an α of 0.05 and 1-ß of 0.80, a sample of at least 10 subjects per group was considered desirable.

Pre-operative work-up

A complete examination of the oral hard and soft tissues was carried out for each patient. Panoramic radiographs and, where necessary, computed tomography scans were undertaken Pre-operative work-ups included an assessment of the edentulous mandible using casts and diagnostic wax-up. A set-up of teeth in wax was done and a surgical template was prepared for each case. A cross-arch acrylic temporary empty shell was also prepared for the total edentulous patients.

Dental implant and surface characterization.

Screw-shaped implants (Black Fix, Titanium Fix, Sao Jose dos Campos, SP, Brazil) made of grade-4 titanium were blasted with aluminum oxide (Al_2O_3) particles (100µm) and washed with nitric acid (HNO_3) solution. The measured parameters, such as the arithmetic average of all profile point absolute values (Ra), the root-mean-square of all point values (Rq), and the average absolute height values of the five highest peaks and the depths of the five deepest valleys (Rz) were 0.74 ± 0.07µm, 0.95 ± 0.06µm and 3.08 ± 0.94µm respectively. This implant system uses a cone Morse tapper connection associated with an indexed double internal hex. The cone Morse presents a tapper angle of 11^{o} **(Figure 1).**

Implant placement

Local anaesthesia was obtained by infiltrating articaine 4% containing 1:100.000 adrenaline. An extended crestal incision was made, with or without releasing incisions, and full thickness flaps were elevated exposing the alveolar ridge. When indicated, a flattening of the alveolar crest was performed with a bur, under irrigation with sterile saline, in order to obtain a larger and flat bony base. Four to five implants were placed in each edentulous mandible (in a full-reconstruction of the mandible) or the necessary implants to restore the partially edentulous area. These implants were placed in a 6 months period (April-September 2009). The preparation of implant sites was carried out with twist drills of increasing diameter (2.8mm or 3.0 mm, according the bone density, to place an implant with 3.5 mm diameter), under constant irrigation. Implants were positioned at the bone crest level. Care was taken to assess the position of the mental foramen.

The prosthetic abutments were inserted immediately after implant placement with 25N/cm of torque. The flaps were then repositioned and were secured around the abutments by interrupted sutures.

Restorative procedures

Immediately following implant surgery, the impression posts were tightened into the abutments. An auto-polymerizing pattern resin was used to connect the impression posts to each other (fully edentulous mandible) to the surgical template. In single implant-supported restoration, the impression post was connected only in the acrylic surgical template.

An impression was taken utilizing a silicon putty polyvinilsyloxane directly on the

impression posts. Laboratory abutment analogs were attached to the modified surgical template (surgical template with resin fixed impression posts) and a master cast was fabricated.

Pre-fabricated implant components and bars made of titanium were welded directly on the master cast by the laboratory technician. The bar was fabricated in an L-shape for strength, and cantilever length was planned according to anterior-posterior spread protocol. Passive fit was obtained and verified using the one-screw test and visual observation. Acrylic reinforced prosthesis with a titanium framework with 12 teeth was delivered within 48 hours. Partially edentulous patients also received an acrylic titanium reinforced implant supported restoration.

The implant-supported restoration was placed over the abutments. Screws were tightened according the manufacturer's instructions. All centric and lateral contacts were assessed by an articulating paper and adjusted if necessary. The screw access was then covered with light-cured provisional resin.

At the time of implant supported restoration delivery, a panoramic radiograph was made to check implant position and the coupling between prosthetic components.

Post-operative treatment

All patients received oral antibiotics (Clindamicyn, 900mg each day) for 7 days Postoperative pain was controlled by administering 100 mg nimesulide every 12 hours for 5 days. Detailed oral hygiene instructions were provided, with mouth-rinses with 0.12% chlorhexidine administered for 7 days. Suture removal was performed at 7 days. After surgery, the patients were instructed to avoid brushing and any trauma to surgical site. A cold and soft diet was recommended for the first day, and a soft diet for the first week.

The patients were scheduled for weekly control visits during the first month. During

each visit, prosthetic functionality and tissue healing were evaluated.

Short–term follow-up and clinical examination

At the 4-month follow-up visit, the implant-supported restoration was removed and the stability of each implant was tested with the pressure of two opposing instruments. Then, clinical parameters such as presence (1) or absence (0) of plaque, gingival bleeding, bleeding on probing, suppuration and measures of PD (mm) and CAL (mm) were determined at six sites per implant (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) by the PD and CAL measurements were recorded to the nearest millimeter using a North Carolina periodontal probe.

All clinical examinations were performed by two (T.O and K.C.S.A) calibrated[11] examiners. The inter-examiner variability was 0.20mm for PD and 0.2mm for CAL. For the first examiner (T.O), the intra-examiner mean SE variability was 0.1mm for PD and 0.2mm for CAL. The second examiner (K.C.S.A) presented a mean SE variability of 0.18mm and 0.25mm for PD and CAL, respectively. These trained examiners were able to provide reproducible measures below 0.5mm. The periodontal parameters registered dichotomously, i.e., plaque accumulation, gingival bleeding, bleeding on probing and suppuration, were calculated in the same way, with two different evaluations by the k-light test ($p<0.05$), which takes into account the contribution of agreement by chance. The inter-observer agreement ranged between 0.8 and 0.95, while the intra-observer agreement was between 0.88 and 0.97 for the first examiner (T.O) and 0.78 and 0.88 for the second examiner (K.C.S.A).

Standardized intra-oral periapical radiographs were obtained using a dental X-ray machine equipped with a 35-cm-long cone. Exposure parameters were 70 kV (peak),

15mA and 1/4 s at a focus-to-sensor distance of 30 cm. The radiographs were captured with a digital camera and transferred to a personal computer. Image processing software was used to store the digitized images. Subsequently, the images were displayed on a monitor and linear measurements were taken with software (Image J 1.4o/java 1.6.0_07 software - Wayne Rasband National Institutes of Health, USA http://rsb.info.nih.gov/ij). The linear distance in millimeters between the implant shoulder and the first clear bone-to-implant contact, mesially and distally, were recorded. The mesial and distal surface values were averaged. A blinded trained examiner (L.A.G.C) performed all radiographic analysis twice.

Implant Success

The evaluation of implant success was adapted from a previous report.[12] To achieve implant success, the following adapted clinical and radiographic success criteria should be fulfilled:

- absence of pain or sensitivity upon function
- absence of suppuration or exudation
- absence of clinically detectable implant mobility
- absence of continuous peri-implant radiolucency
- distance between implant shoulder and the first visible radiographic (DIB) < 1.0 mm after 4 months of functional loading

Immunological assessment of PICF samples

PICF samples were taken at baseline (7 days after loading) and 4 months follow-up using sterile standardized paper strips (PerioPaper, Oraflow, Smithtown, NY). The samples at baseline were taken only after 7 days follow the surgery to allow a peri-implant sulcus formation, at least a anatomic configuration. Following the isolation of the sampling area with sterile cotton rolls, supragingival plaque was removed, and the site was air-dried gently to reduce any contamination with plaque and/or saliva. Paper strips were inserted to a standardized depth (~ 2mm) at mesio-buccal site, regardless of the probing depth, and a standard sampling time (30 seconds) was used. These measures were considered to be necessary for the standardization of the sampling procedure. Samples with evidence of bleeding were not included. To eliminate the risk for evaporation, paper strips with PICF were transported immediately to a chair-side electronic gingival fluid measuring device (Periotron 8000, Proflow, Amityville, NY) that had been previously calibrated. The electronic PICF volume was measured with the device, and the units were converted to microliters by a software program. Throughout the experimental period, the reliability of the calibration of the device was checked at periodic intervals and, when necessary, it was renewed by triplicate readings. After electronic volume determination, PICF samples were placed in sterile microtubes that were stored at -80C until the day of laboratory analysis.

PICF samples were analyzed by ELISA for sRANKL and OPG using commercially available ELISA kits (Biomedica Medizinprodukte, Wien, Austria.). The strips were suspended with buffer-phosphate solution into the tubes and vortexed for 30 seconds and centrifuged for 5 minutes at 1,500 x g to elute. Assays were carried out according to the manufacturer's recommendations using human recombinant standards. One hundred microliters and 50 ul of each sample were added in each well for RANKL and

OPG assays, respectively. The OPG coating antibody was a monoclonal anti-OPG, whereas the OPG detection antibody was a goat polyclonal biotinylated anti-human OPG. For RANKL analysis, the microtiter strips were coated with human recombinant OPG, and the RANKL detection antibody was a goat polyclonal biotinylated antihuman sRANKL. The optical density was measured at 450 nm. The absorbance readings were converted to the amounts of RANKL or OPG per well using a trendline equation that was prepared based on the readings of the standard curve supplied by the manufacturer. The concentrations of the recombinant standard ranged from 0 to 6 pmol/l and from 0 to 30 pmol/l for RANKL and OPG, respectively. The negative controls for both assays were the PBS without PICF samples. Results were reported as the total amounts (in picograms) of sRANKL and OPG per site in 30 seconds. Sites with sRANKL or OPG levels below the detection limit of assay were scored as 0 pg. The calculation of sRANKL and OPG concentrations in each PICF sample (in picograms per microliter) was established by dividing the total amount of each protein by the volume of fluid.

Statistical analysis

Mean and standard deviation of the clinical parameters and cytokines levels were calculated for each implant and then for each group and periods. Differences between the periods and between the groups were evaluated using Wilcoxon test and Mann-Whitney test ($p<0.05$) respectively.

RESULTS

Patient population and implants distribution

The demographic data of the patients at baseline was presented in Table 1. Twenty-two patients were evaluated at the end of the study due to the drop-outs of two patients. A total of 88 dental implants were evaluated (Table 2).

Clinical evaluation and Implant Success

There were 2 patients who dropped out from this study and only 88 implants were examined at the 4-month recall. Three implants in 3 different patients from control group presented lack of osseointegration at 4-month follow-up. These failures were attributed to lack of osseointegration/occlusal overload, without clinical signs of peri-implant infection (suppuration). Therefore, 85 (96.59%) dental implants were successfully osseointegrated at the end of the study (Table 2).

Eight-five implants were still in function at 4-month study. Among these implants, 100% were classified in the implant success group. All these implants did not show pain or clinical mobility, suppuration or exudation, with a DIB <1.5 mm. The overall radiographic evaluation of the bone loss/remodeling around immediately loaded implants revealed a mean of 0.66 mm (± 0.4) at the 4-months examination. Clinical parameters were presented in Table 3. All clinical parameters were similar in both groups (p>0.05).

Prosthetic complications and maintenance

Prosthetic complications included result of cracked or fractured restoration (1 patient in control group, 4.3%).

Volume of PICF, RANKL and OPG levels

Volume (microliter) of PICF, total amounts (picograms per site) and concentrations (picograms per microliter) of sRANKL and OPG of patients with and without osteopenia at baseline and 4 months loading were presented in Figures 2 and 3, respectively.

The PICF volume fluctuated. The baseline PICF volume was significantly higher than at baseline ($p<0.05$), and the decrease at 4 months was significant ($p<0.05$).

Total amounts and concentrations of sRANKL, OPG and ratios of RANKL/OPG were not significantly different between groups ($p<0.05$) in both baseline and 4 months. However, there were differences between baseline and 4 months period for both groups. The amount and concentration of RANKL were higher at baseline when compared with 4 months period ($p<0.05$). OPG levels in PICF (total volume and concentration) increased significantly after 4 months of loading ($p<0.05$). In addition, the RANKL/OPG ratios decreased after 4 months period ($p<0.05$).

DISCUSSION

This study focused to evaluate the osteoclastogenesis-related factors (RANKL and OPG) levels in the peri-implant crevicular fluid (PICF) of immediately loaded implant with platform switching in patients with and without osteopenia after 120 days of loading**.** The preliminary outcomes for the treated patients indicated that osteopenia did not influence the prognosis of implant success ratio neither the levels of the RANKL and OPG in the PICF, at least in the short term.

At baseline, the surgical trauma associated with the loading of the implants generated a unique microenvironment around the implant, with higher means of RANKL levels in

PICF of both groups, suggesting an osteoclast formation and bone resorbing activity. At 4 months follow-up, the OPG levels increased significantly ($p<0.05$), showing remodeling process activity. Thus, it is the balance between the expression of RANKL and OPG that determines the extent of bone resorption. These data, together, might reflect bone turnover rather than the inhibition of bone resorption.

As the expression of the OPG increases, a higher bone formation can be induced due to the prohibition of the formation of osteoclast. As mentioned before, the expression ratio of RANKL and OPG is an important parameter for controlling the absorption of bone, and it plays an important role in controlling the degrees of the bone absorption or bone mineral density by effecting the amount of the RANK available for osteoclast. Therefore, in this investigation, the level of OPG compared to the level of RANKL is an important factor in estimating the effect of osteopenia on peri-implant bone formation. Based on the results of this investigation, OPG was expressed since baseline in both groups. By fourth month, the level of OPG increased and the RANKL decrease, suggesting a balance between bone resorption and bone apposition.

The importance of the RANK/RANKL/OPG pathway in the formation of osteoclasts has been clearly demonstrated,[13-14] however, osteopenic patients presented the same levels of cytokines of control patients, suggesting that the bone turnover at peri-implant environment was not affected. In subjects with osteopenia, the decreases net bone volume, as well as the reduced withstanding optimal load may be affected by a combination of these modulated cellular activities influenced by lower levels of estrogen in post-menopausal osteopenia.[15] In addition, could be speculated that bone-to-implant integration gradually increases and, once it is established, the accumulated rate of bone-attaching to implants is maintained.[3] Unlike regular bone remodeling

occurring in the trabecular area, this phenomenon is not accompanied by apparent turnover or resorption bone.[16]

The overall 96.59% implant success rate for the immediate loading was comparable with previous studies performed in patients without osteopenia/osteoporose.[17-19] The clinical data also ratify the idea that immediate loading protocols have the same influence on both groups. The bone remodeling at crestal bone level presented an average of 0.72mm and 0.61mm to control and osteopenic patients respectively. The implant-supported prostheses in jawbone are affected not only by systemic factors, but also by many local factors such as periodontal conditions of the remaining teeth, number and distribution of dental implants in the arch, occlusion and bite forces. Although several studies relate the role of local and systemic factors in the long-term success of dental implants, less is know concerning factors affecting the stability of oral implants after abutment placement process and occlusal load.[20] Therefore, the role of endogenous factors on cellular turn over and differentiation is still less documented.[21]

Systemic conditions associated with osteoporosis and osteopenia have been postulated to contribute to the severity of alveolar bone loss.[22] The concept that dental implant placement might be contraindicated in subjects with osteoporosis/osteopenia is based on the assumption that these pathologies may affects the human jaws in the same way that other parts of the skeleton. Complementary, there may be some differences in bone healing and remodeling between the long bones and the jawbones after dental implant placement.[15,21] However, to date, there are not conclusive studies presenting that osteoporosis and/or osteopenia are absolutely contraindicated for dental implants placement.

CONCLUSION

Within the limits of this study, it could be suggested that osteopenia did not influenced the peri-implant tissue response to the osteoclastogenesis factors in PICF of immediately loaded implants, at least, after 120 days post-surgery.

Acknowledges

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Figure Legends

Figure 1: A) Schematic drawing of the implant system evaluated; **B)** Scanning electron microscopy of the implant surface topography; **C)** Atomic force microscope analysis of the surface topography.

Figure 2. Distribution of **a)** volume (microliters), total amounts (picograms per site per 30 seconds) of **b)** sRANKL, **c)** OPG, **d)** RANKL/OPG ratio as well as the concentrations (pg/ml) of **e)** sRANKL and **f)** OPG in the PICF of subjects with and without osteopenia at baseline and 4 months loading. Horizontal lines show median and sem values. The individual symbols represent the total amount at each implant. *Differences between baseline and 4 months loading (Wilcoxon test; $p < 0.05$); ns – non-significant differences between groups (Mann-Whitney Test, $p > 0.05$).

Table 1 – Average (+SD) evaluation of demographic data of the subjects of both groups. *Mann-Whitney Test*: *$p < 0.05$.

	Control	**Osteopenia**
n	10	13
Age (years)	61.81±5.26	61.50±6.60
DXA*	0.17±0.71	-1.52±0.31
Edentulous		
Totaly	3	5
Partialy	7	8
Full-mouth data of the remaining teeth		
PD (mm)	2.23±0.98	1.77±0.51
CAL (mm)	1.15±1.0	1.26±1.1
% sites		
PI	13.82±3.82	7.8±2.33
GI	3.58±1.28	2.32±1.96
BOP	11.40±6.71	10.25±6.11
SUP	0	0

DXA: dual energy X-ray absorptionmetry; **PD:** Pocket depth; **CAL:** clinical attachment level; **PI:** plaque index; **GI:** gingival index; **BOP:** bleeding on probing; **SUP:** suppuration.

Table 02: Distribution, position and length (3.5mm diameter) of the implants used in the study. The numbers of the teeth are according to international classification.

GROUP	NUMBER OF IMPLANTS	LENGTH(mm)	POSITION OF IMPLANTS													
			47	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**
CONTROL	38	10		1											3	
		11.5		2	2	2		1	2	3			2	2	2	1
		13			1	4		1					4	1	3	1
		15														
OSTEOPENIA	50	10				1									2	
		11.5		2		2	1		2	2			3	2	2	
		13	1	2	1	2	1		2	2		2	1	1	3	
		15		2	2	2			2	2			3			
	TOTAL		1	9	6	13	2	2	8	9	0	2	13	6	15	2

Table 3 – Mean±sd of the clinical parameters of the implants after 120 days post-therapy, for both groups. *Mann-Whitney Test* $p > 0.05$.

	Control	**Osteopenia**
PD (mm)	3.37±0.71	3.94±1.63
CAL (mm)	2.51±0.45	2.42±0.57
BL (mm)	0.72±0.28	0.61±0.52
% Site		
GI	8.37±15.45	6.15±15.77
PI	3.12±5.78	0
BOP	17.39±45.03	16.01±33.02
SUP	0	0

PD: Pocket depth; **CAL:** clinical attachment level, **BL:** radiographic bone loss; **GI:** gingival bleeding; **PI:** plaque index; **BOP:** bleeding on probing; **SUP:** suppuration .

Figure 1

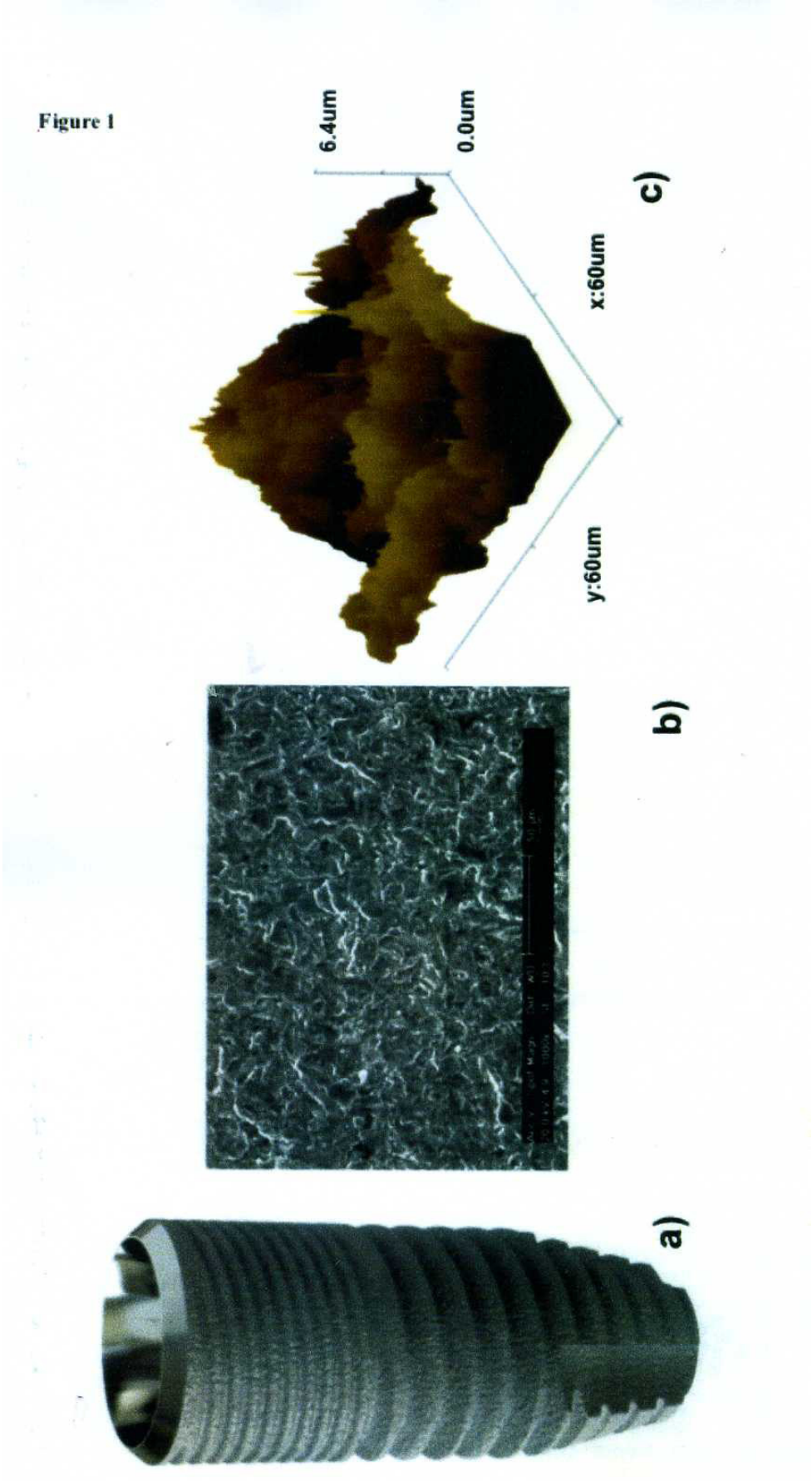
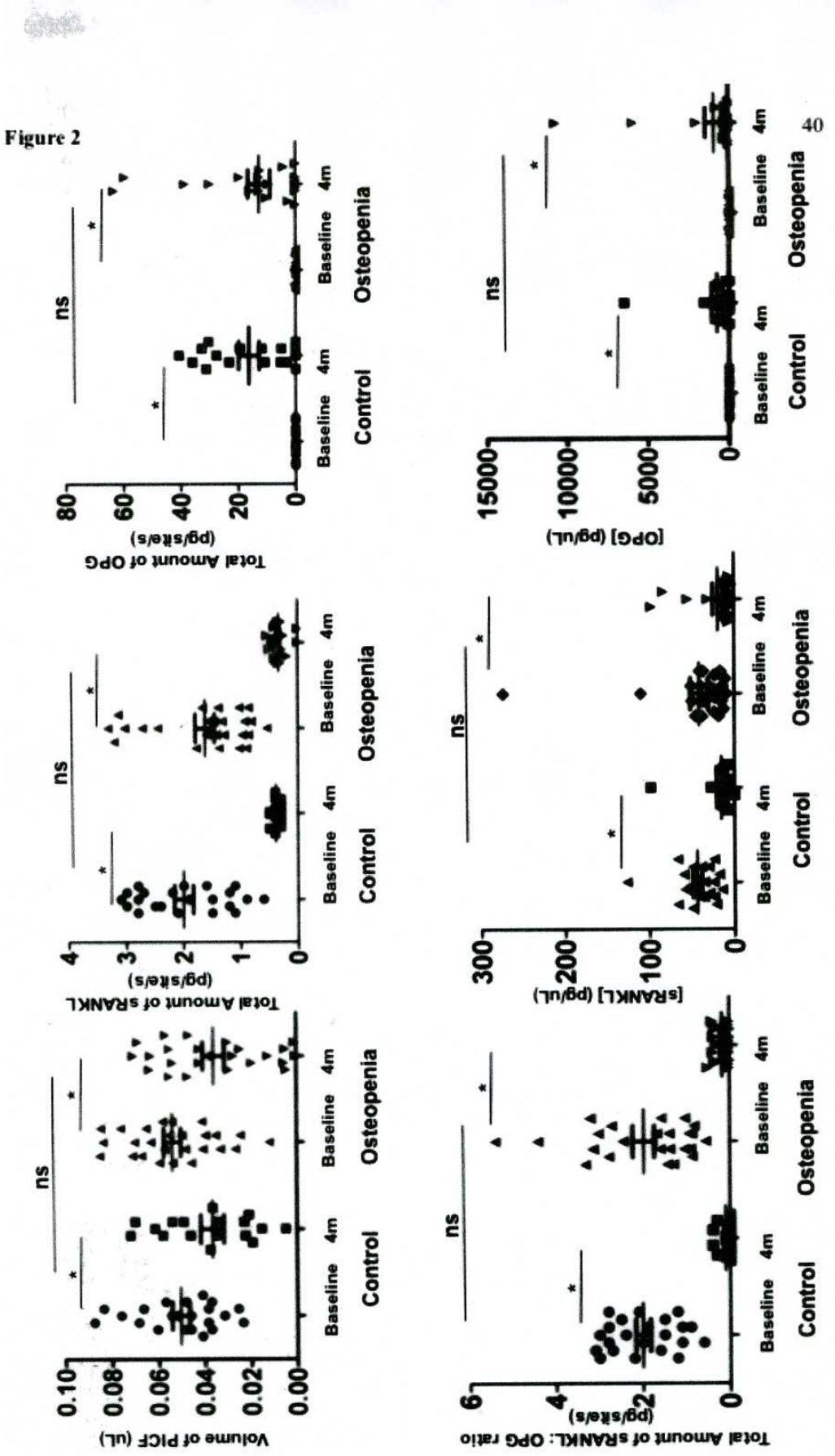


Figure 2


Total Amount of OPG (pg/ste/s)
[OPG] (pg/uL)
Total Amount of sRANKL (pg/ste/s)
[sRANKL] (pg/uL)
Volume of PICF (uL)
Total Amount of sRANKL: OPG ratio (pg/ste/s)
ns
*
Baseline
4m
Control
Osteopenia

40

4. CONCLUSÃO

Dentro das limitações deste estudo, pode ser sugerido que a osteopenia não influenciou os parâmetros clínicos e na resposta dos fatores de osteoclastogênese (RANKL e OPG) no fluido crevicular dos tecidos per-implantares dos implantes imediatamente carregados, pelo menos, depois de 120 dias de pós-cirurgia.

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