PONTIFÍCIA UNIVERSIDADE CATÓLICA DE MINAS GERAIS
Programa de Pós-graduação em Odontologia

Carla Dias Matos

# AVALIAÇÃO DE PARÂMETROS CLÍNICOS E DA MMP-8 EM IMPLANTES E DENTES HOMÓLOGOS

Belo Horizonte
2014

Carla Dias Matos

# AVALIAÇÃO DE PARÂMETROS CLÍNICOS E DA MMP-8 EM IMPLANTES E DENTES HOMÓLOGOS

Dissertação apresentada ao Programa de Pós-graduação em Odontologia da Pontifícia Universidade Católica de Minas Gerais, como requisito parcial para obtenção do título de Mestre em Odontologia - Área de Concentração: Implantodontia.

Orientador: Prof. Dr. Rodrigo Villamarim Soares
Coorientador: Prof. Dr. Elton Gonçalves Zenóbio

Belo Horizonte
2014

FICHA CATALOGRÁFICA
Elaborada pela Biblioteca da Pontifícia Universidade Católica de Minas Gerais

Matos, Carla Dias
M433a Avaliação de parâmetros clínicos e da MMP-8 em implantes e dentes homólogos / Carla Dias Matos. Belo Horizonte, 2014.
45 f. : il.

Orientador: Rodrigo Villamarim Soares
Coorientador: Elton Gonçalves Zenóbio
Dissertação (Mestrado) – Pontifícia Universidade Católica de Minas Gerais. Programa de Pós-Graduação em Odontologia.

1. Implantes dentários - Análise. 2. Biofilmes. 3. Colagenase de neutrófilo. 4. Periodontite. I. Soares, Rodrigo Villamarim. II. Zenóbio, Elton Gonçalves. III. Pontifícia Universidade Católica de Minas Gerais. Programa de Pós-Graduação em Odontologia. IV. Título.



CDU 616.314-089.843

**Carla Dias Matos**

# AVALIAÇÃO DE PARÂMETROS CLÍNICOS E DA MMP-8 EM IMPLANTES E DENTES HOMÓLOGOS

Dissertação apresentada ao Programa de Pós-graduação em Odontologia da Pontifícia Universidade Católica de Minas Gerais, como requisito parcial para obtenção do título de Mestre em Odontologia. Área de Concentração: Implantodontia.

**COMPOSIÇÃO DA BANCA EXAMINADORA:**

1- Profa. Dra. Luciene Cristina de Figueiredo – UnG
2- Prof. Dr. Maurício Greco Cosso – PUC Minas
3- Prof. Dr. Rodrigo Villamarim Soares – PUC Minas

**DATA DA APRESENTAÇÃO E DEFESA: 28 de julho de 2014**

**A dissertação, nesta identificada, foi aprovada pela Banca Examinadora**

Belo Horizonte, 18 de agosto de 2014

Prof. Dr. Rodrigo Villamarim Soares
**Orientador**

Prof. Dr. Martinho Campolina Rebello Horta
**Coordenador do Programa de Pós-graduação em Odontologia**

**Ao Joviano, meu esposo, amigo companheiro que sempre acreditou neste projeto e me apoiou de forma incondicional e,**
**ao Bernardo meu filho, amo vocês!**
**Ao meu pai, Edmar, pelo exemplo, ensinamento, apoio incondicional e amor sempre dedicado.**
**À minha mãe, Luiza, pelo amor, torcida, ajuda e dedicações com meu filho Bernardo em todos os momentos que precisei amo você mãe.**
**A vocês, dedico este trabalho!**

## AGRADECIMENTOS

**A Deus** por permitir que eu usufrua da beleza que é o exercício desta profissão, por me ensinar que através da fé tudo é possível e por colocar pessoas tão especiais e iluminadas em minha vida.

Aos **meus irmãos**, Mateus e Vanessa, e ao Henrique, pela amizade e companheirismos. Agradeço pelo apoio.

Ao meu orientador, **Prof. Rodrigo Villamarim Soares,** pelo apoio, incentivo para realizar este trabalho. Minha profunda admiração e agradecimento por sua disponibilidade, pelo conhecimento transmitido e pela amizade demonstrada nos momentos desta caminhada.

Ao **Professor e co-orientador**. Dr. Elton Gonçalves Zenóbio, pele confiança, respeito, oportunidades, ensinamentos transmitidos. Obrigada pela oportunidade.

À **Maria Cândida**, colega do mestrado, que ajudou e compartilhou suas experiências na parte da coleta de dados desta pesquisa e ao longo de todo o curso, tornando-se uma grande amiga.

Aos **professores** do Mestrado profissional: Mauricio Greco, José Alfredo Mendonça, Peterson Dutra, Antônio Henrique, Paulo Nascimento e Emílio Akaki, pelos ensinamentos transmitidos, momentos de convívio e amizade.

A todos os **professores** e **funcionários** do Departamento de Odontologia da PUC Minas que de alguma forma contribuíram com a minha formação, e em especial ao professor **João Batista** pelo apoio.

Aos **amigos** do mestrado, Maria Cândida, Hugo Araújo, Bruno Ramos, Pedro Henrique e Leandro Junqueira, pelos conhecimentos compartilhados, pela ajuda recíproca e amizade ao longo desta caminhada e momentos tão agradáveis.

À **Andreia Cruz**, que sempre ajudou e me deu suporte em tantos momentos.

Aos **pacientes** que participaram desta pesquisa.

## RESUMO

A avaliação da condição perimplantar e periodontal pode ser regularmente realizada por meio de parâmetros clínicos e radiográficos. Os objetivos do presente estudo foram avaliar e correlacionar parâmetros clínicos perimplantares e periodontais, e a expressão da metaloproteinase de matriz 8 (MMP-8) em sítios perimplantares e periodontais de implantes e dentes homólogos assim com entre implantes com conexão externa (HE) e interna (HI). Foram selecionados 47 pacientes que não apresentavam doença perimplantar ou periodontite, nestes foram avaliados 79 implantes (48 HE; 31 HI) com prótese instalada há pelo menos 6 meses, e 79 dentes. A profundidade de sondagem (PS), faixa de mucosa ceratinizada (FMC) e espessura da mucosa ceratinizada (EMC) foram avaliadas. O fluido crevicular perimplantar e o gengival foram coletados e quantificados (Periotron®), e nestes a expressão da MMP-8 foi avaliada (ELISA). A PS dos implantes foi significativamente superior a dos dentes, e a FMC significativamente inferior. A concentração da MMP-8 em implantes foi significativamente superior a dos dentes. Correlações entre a expressão da MMP-8 e a PS, FMC, EMC em implantes e dentes, assim como em HE e HI não foram observadas. A frequência da distribuição de FMC e EMC em faixas específicas, assim como da expressão da MMP-8 nestas faixas não revelou diferenças significativas entre implantes e dentes, assim como entre HE e HI. Uma significativa correlação negativa entre o volume dos fluidos perimplantares e gengivais com a expressão da MMP-8 foi observada em implantes e dentes, assim como em HE e HI. A maior PS (2 mm) e menor FMC (2 mm) observada nos implantes possui pequeno significado clínico. A maior expressão da MMP-8 nos implantes não foi associada aos parâmetros avaliados. Portanto, implantes e dentes clinicamente saudáveis, assim como implantes com diferentes plataformas apresentam parâmetros clínicos similares assim como concentração de MMP-8.



**Palavras-chave:** Implantes. Parâmetros clínicos. Fluido crevicular. MMP-8.

## ABSTRACT

The evaluation of the perimplantar and periodontal condition can be regularly performed by means of clinical and radiographic parameters. The present study aimed to evaluate and correlate the perimplantar and periodontal clinical parameters and the matrix metalloproteinase-8 (MMP-8) expression in perimplantar and periodontal sites of implant and homologous teeth, as well as between external hexagon (EH) and internal hexagon (IH) implants. This study selected 47 patients who presented no perimplantar disease or periodontitis. In these 47 patients, 79 dental implants (48 EH, 31 IH), in which the prosthesis had been installed at least six months prior, and 79 HT were evaluated. The probing depth (PD), width of keratinized mucosa (WKM), and thickness of keratinized mucosa (TKM) were evaluated. The perimplantar and gingival crevicular fluid were collected and quantified (Periotron®), and in these, the MMP-8 expression was evaluated (ELISA). The PD of the implants was significantly higher than that of the HT, while the WKM was significantly lower. The concentration of the MMP-8 in the implants was significantly higher than those of the HT. Correlations between the MMP-8 expression and the PD, WKM, and TKM in the dental implants and the HT, as well as in the EH and IH, were be observed. The distribution rate of the WKM and TKM in specific categories, as well as the MMP-8 expression in these same categories, presented no statistically significant differences among the HT, nor between the EH and IH. One significant negative correlation between the perimplantar and gingival fluids and the MMP-8 expression was observed in the dental implants and HT, as well as in the EH and IH. The largest PD (2 mm) and the smallest WKM (2 mm) observed in the dental implants showed little clinical significance. The higher MMP-8 expression in the dental implants proved not to be associated with the evaluated parameters. Therefore, the dental implants and the clinically healthy HT, as well as the dental implants with different platforms and the MMP-8 concentration, presented similar clinical parameters.



**Keywords:** Dental implants. Clinical parameters. Crevicular fluid. MMP-8.

## LISTA DE ABREVIATURAS

HE – Hexágono Externo

HI – Hexágono Interno

DH – Dentes Homólogos

PS – Profundidade de Sondagem

EMC – Espessura da Mucosa Ceratinizada

FMC – Faixa de Mucosa Ceratinizada

FCG – Fluido Crevicular Gengival

FCPI – Fluido Crevicular Periimplantar

VFC – Volume do Fluido Crevicular

**SUMÁRIO**

## 1 INTRODUÇÃO

A disseminação da implantodontia aumentou também o índice de insucesso, que de acordo com o relatório do Consenso do Sexto Workshop Europeu em Periodontia, está relacionado à presença da doença perimplantar. A mucosite perimplantar (inflamação restrita a mucosa perimplantar) é relatada em 80% dos indivíduos com implantes, e a perimplantite (inflamação perimplantar com perda óssea adicional) em até 56% dos casos (MONTES et al., 2009).

A periodontite é uma doença de etiologia bacteriana, decorrente de um desequilíbrio entre a agressão provocada pelos microrganismos e a resposta da defesa do hospedeiro. Este desequilíbrio é provocado pela exacerbação da resposta inflamatória e imunológica, frente à ação dos microorganismos presente no biofilme dental. O biofilme aderido a superfície do dente ativa uma reação inflamatória intensa, gerando uma degradação da matriz extracelular levando a reabsorção do osso alveolar (BAKER, 2000; KINANE et al., 2001).

A perimplantite é provocada pela presença de reações inflamatórias que afetam os tecidos perimplantares. Os sinais variam desde uma inflamação restrita a mucosa ao redor dos implantes até sangramento à sondagem, supuração, perda inserção avaliada clinicamente, e perda óssea em formato de taça observada radiograficamente (MOMBELLI, 1999).

O desenvolvimento de analises imunológico e diagnóstico microbiológico auxilia no exame mais detalhado da composição do biofilme bacteriano supra e subgengival, e para uma avaliação mais eficaz do efeito das terapias periodontais e perimplantares (GRABER et al., 1999; BOOTH; LEHNER, 1997).

Os biomarcadores são uma ferramenta no auxilio de diagnóstico da doença periodontal e perimplantar. Os biomarcadores presente na saliva e no fluido crevicular gengival são indicadores da saúde fisiológica, do processo patogênico e da resposta do indivíduo.

Na resposta inflamatória dos tecidos periodontais e perimplantares apresentam citocinas como a prostaglandina E2 (PGE2), interleucinas (IL)- 1β, IL6 e fator de necrose tumoral (TNF), que são liberados pelo epitélio juncional das células como os fibroblastos, macrófagos e polimorfonucleares (PMN). As enzimas como metaloproteinase de matriz (MMP-8, MMP-9 e MMP-13), são produzidas pelos PMN

e osteoclastos levando a degradação do colágeno e do osso alveolar (TABA JÚNIOR et al., 2005).

Mediadores presentes no fluído crevicular funcionam como indicadores da presença de bactérias Gram-negativas ativando os macrófagos que liberam citocinas e mediadores inflamatórios, que estimulam células osteoclásticas resultando em perda óssea (NGUYEN et al., 1991; RASMUSSEN et al., 2000).

A resposta imunológica é diferente entre o tecido perimplantar e o tecido periodontal, principalmente na produção de algumas citocinas que induzem a degranulação de neutrófilos, e a liberação de colagenase tipo 2 e de matriz de metaloproteinase 8 (MMP-8) (SORSA et al., 1999).

As enzimas metaloproteinase de matriz (MMP) são enzimas proteicas responsáveis pela degradação e remodelação tecidual encontradas com maior prevalência no fluido crevicular gengival (FGC) e em sítios com doença periodontal. A MMP-8 inicia a degradação do colágeno Tipo I, que é o mais encontrado nos tecidos periodontais. Estudos demostram que a MMP-8 é a principal colagenase intersticial no FGC em periodontite crônica, e no fluido sulcular periimplantar em perimplantite (SORSA et al., 1999).

A expressão e a atividade da enzima metaloproteinase 8 (MMP-8) é baixa ou limitada nos sítios periodontais e perimplantares saudáveis. Entretanto, em várias condições patológicas podem ocorrer o aumento significante da MMP-8 ocasionando uma destruição tecidual, processos inflamatórios, crescimentos tumorais e metástase (SORSA et al.,2004). Alguns estudos têm demostrado que a MMP-8 e a colagenase tipo 2 são as mais prevalentes sendo encontradas em elevadas concentrações na doença periodontal (SORSA et al., 2004).

Com a utilização dos implantes na reabilitação das áreas edêntulas, ocorreu a necessidade de desenvolver estudos para analisar os marcadores bioquímicos da inflamação utilizando o fluido crevicular perimplantar como uma ferramenta contemporânea de diagnóstico de saúde ou não da região, assim como dos parâmetros clínicos para estabelecer os riscos de destruição perimplantar nos sistemas de conexão externa e interna.

# 2 OBJETIVOS

## 2.1 Objetivo geral

Avaliar e correlacionar parâmetros clínicos perimplantares e periodontais, e a expressão da MMP-8 em sítios perimplantares com conexão externa e conexão interna e periodontais homólogos.

## 2.2 Objetivos específicos

a) comparar os parâmetros clínicos profundidade de sondagem (PS), faixa da mucosa ceratinizada (FMC), espessura da mucosa ceratinizada (EMC) e volume do fluido crevicular (VFC) de implantes e dentes homólogos, assim como entre implantes com sistema de conexão externa (hexágono externo) e conexão interna (hexágono interno e cone morse);
b) comparar os níveis da enzima MMP-8 de sítios perimplantares e periodontais homólogos, assim como entre implantes com sistema de conexão externa e interna;
c) avaliar possíveis correlações entre PS, FMC, EMC e VFC em implantes e dentes homólogos, assim como entre implantes com sistema de conexão externa e conexão interna com os níveis da MMP-8.

## 3 ARTIGO

### Análise de parâmetros clínicos e da expressão da mmp8 em implantes e dentes homólogos

Artigo foi formatado de acordo com as normas de publicação do periódico: Clinical Oral Implants Research (Qualis: A2).

Normas para submissão de artigos podem ser visualizadas no endereço eletrônico: http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1600-0501/issues.

# Analysis of the clinical and MMP-8 expression parameters in tooth implants and homologous teeth

Carla Dias Matos[1]
Gabriela Giro[2]
Sergio Luis Scombati de Souza[3]
Jamil Awad Shibli[2]
Rodrigo Villamarim Soares[4]
Elton Gonçalves Zenóbio[4]

[1] Graduate Student Master Dental Implant Program, Department of Dentistry, Pontific Catholic University of Minas Gerais (PUC Minas), Belo Horizonte, Brazil.
[2] Department of Periodontology and Oral Implantology, Dental Research Division, University of Guarulhos.
[3] Adjunct Professor, University of São Paulo, School of Dentistry of Ribeirão Preto, Department of BMFS e Periodontology.
[4] Adjunct Professor, Master Dental Implant Program , Department of Dentistry, PUCMinas, Belo Horizonte, Brazil.

**Corresponding address:**
Rodrigo Villamarim Soares
Pontifícia Universidade Católica de Minas Gerais
Programa de Pós-graduação em Odontologia
Av. Dom José Gaspar, 500, Prédio 46, Sala 101
Belo Horizonte, MG, Brasil, 30535-901
Phone: 55 - 31- 3319-4414, Fax: 55 - 31- 3319-4415
E-mail: soaresrv@pucminas.br

## Abstract

**Background:** The evaluation of the perimplantar and periodontal condition can be regularly performed by means of clinical and radiographic parameters. The present study sought to evaluate and correlate the perimplantar and periodontal parameters and the metalloproteinasis-8 (MMP-8) matrix in perimplant and homologous periodontal sites (HT) as well as in external hexagonal (EH) and internal hexagonal (IH) implants. **Materials and Methods:** Forty-seven patients without perimplantar disease or periodontitis were selected for this study. Of these 47 patients, 79 implants (48 EH, 31 IH), with prosthesis installed for at least six months, and 79 HT were evaluated. The probing depth (PD), width of keratinized mucosa (WKM), and thickness of keratinized mucosa (TKM) were evaluated. The perimplantar and gingival crevicular fluids were collected and quantified (Periotron®) and the MMP-8 expression was evaluated (ELISA). **Results:** The PD of the implants was significantly higher than that of the HT, while the WKM was significantly lower. The concentration of MMP-8 in the implants was significantly higher than that of the HT. Correlations between the MMP-8 expression and the PD, WKM, and TKM in the implants and the HT, as well as in the EH and IH, could be observed. The distribution rate of the WKM and TKM as well as the MMP-8 expression in specific categories, presented no statistically significant differences between implants and HT nor between EH and IH. One significant negative correlation between the perimplantar and gingival fluids and the MMP-8 expression was observed in implants and HT, as well as in EH and IH. **Conclusion:** The largest PD and the smallest WKM observed in implants show little clinical significance. The higher MMP-8 expression in implants proved not to be associated with the evaluated parameters. Therefore, implants with different platforms and HT were clinically healthy in this study.


**Key words:** dental implants, clinical parameters, crevicular fluid, MMP-8

## Introduction

Evaluations of probing depth (PD), bleeding upon probing (BP), and clinical attachment level (CAL) are commonly used in the re-evaluation of dental implants, considering that they enable the evaluation of the presence of health or disease in perimplantar tissues (Heitz-Mayfield, 2008).

The development of immunological and molecular biology techniques have provided support for detailed exams in the composition of bacterial biofilm, as well as the evaluation of implemented periodontal and perimplantar therapies (Booth & Lehner, 1997; Kinane, 2000; Taba Júnior et al., 2005; Graber et al., 1999). Biofilm attached to the surface of the tooth, activates an intense inflammatory reaction, in turn generating a degradation of the extracellular matrix, leading to alveolar bone resorption (Kinane, 2000).

Biomarkers present in the saliva and in the crevicular fluid have been undergoing evaluation in an attempt to improve the diagnosis of periodontal and perimplantar conditions. The presence of cytokines, such as prostaglandine E2, interleukins (IL-1β and IL-6), tumor necrosis factors, as well as matrix metalloproteinases (MMP-8, MMP-9, and MMP-13) can be identified from the inflammatory response of the periodontal and perimplantar tissues (Taba Júnior et al., 2005).

The MMPs are enzymes responsible for tissue degradation and repair. In particular, an increase in the MMP-8 expression and activity in various pathological conditions which can lead to tissue destruction was previously reported by Sorsa et al. (2004). MMP-8 initiates the degradation of type I collagen, the most common type of collagen found in periodontal and perimplantar tissues. Moreover, this enzyme is the main interstitial collagenase present in the crevicular fluid of individuals with periodontitis and perimplantitis (Sorsa et al., 1999). It is believed that a better understanding of how factors influence the pathogenesis of perimplantar and periodontal diseases could lead to improved new therapeutic proposals.

In the present study, perimplantar connections with EH and IH, periodontal parameters in HT, and MMP-8 expression were evaluated and correlated.

## Materials and methods

**Sample selection**

The present study was approved by the Research Ethics Committee of the Pontific Catholic University of Minas Gerais (PUCMinas) protocol number CAAE 03313512.0.0000.5137.

Initially, 200 patients were examined. Individuals were excluded from this study if they were smokers, diabetics, immunosuppressed, osteoporotics, pregnant, HIV positive, had active periodontitis and perimplantitis, were diagnosed with severe systemic alterations, or had taken antibiotics and/or anti-inflammatory drugs within the past three months.

Individuals were included if they had received external hexagon (EH), internal hexagon (IH; Cone Morse) implants with prosthesis installed for at least six months, as well as a natural tooth in the same position (homologous tooth – HT) in the opposite arch.

**Clinical evaluation**

The periodontal and perimplantar exam to diagnose clinically healthy implants and teeth, without mucositis, perimplantitis, or periodontitis, was performed. The clinical parameters of PD; width of keratinized mucosa (WKM), thickness of keratinized mucosa (TKM), volume of crevicular fluid (VCF); implant exposed threads, mobility and periapical radiographs were evaluated.

The PD was obtained by using a UNC-15 periodontal probe (Hu-Friedy®, Chicago, IL, USA) in the central-vestibular and central-lingual sites. This probe was turned mesially and distally around the same sites in the HT and implants, and the deeper measurement of each side was considered. Measurements $\geq$ 4mm excluded the teeth and implants. The BP was evaluated in the same sites in a dichotomous manner (presence or absence); when present, led to the exclusion of the implant and the HT. The WKM was measured using a UNC-15 periodontal probe, recording the distance between the gingival margin and the mucogingival junction in the central buccal region of the implant or homologous tooth. The TKM was measured in triplicate in the central buccal portion of the implant or HT using a Periodontal Caliper® (Digimess, São Paulo, SP, Brasil), as previously described by Yared et al. (2006).

**Samples of Perimplantar Crevicular Fluid (PICF) and Gingival Crevicular Fluid (GCF)**

The clinically visible biofilm was removed, and the collection sites were isolated with cotton pellets. The gingival tissue was delicately dried with an air blast, and the PICF and GCF were collected, using strips of absorbent paper filters (Periopaper®; Oralflow, New York, NY, USA). The paper strips were introduced into the sulcus until encountering tissue resistance and maintained in the same location for 30 seconds. Samples that had been visibly contaminated by blood were discarded, given that the volume of fluid would not be quantified by the Periotron®, and the sites were reevaluated during a later dental visit. The collected fluid was evaluated using a Periotron® 8000 (Oralflow, PlainView, New York, NY, USA) immediately following collections and, in an attempt to reach the highest level of precision, a polynomial regression on the order of 4 was used to calculate the obtained volumes, as previously described by Chang & Wennström (2013). These paper strips were placed in Eppendorf tubes and stored at -80°C.

**Evaluation of the MMP-8 expression**

To determine the MMP-8 concentration in the PICF and GCF samples, 300 µl of PBS solution with 0.05% bovine serum albumin was added to the tubes containing absorbent paper strips. The tubes were then shaken and left at rest for 40 minutes at room temperature. Next, the tubes were centrifuged at 6,000 RPM for 5 minutes and the strips were removed. The samples were processed using ELISA kits (Human MMP-8 DuoSet, R&D systems, Minneapolis, MN, USA) to quantify the MMP-8, following manufacturer instructions.

**Statistical analysis**

The normality of the data was evaluated using the *Kolmogorov-Smirnov* or *Shapiro-Wilk* tests. The comparison between the two independent samples was carried out by applying *the Mann-Whitney U* test and between two paired samples using the *Wilcoxon* or *Student t* test. The correlations between the MMP-8 levels and the evaluated clinical parameters were tested by applying the *Spearman* rank correlation coefficient. The rates were compared by applying *Chi-squared* or *McNemar* tests. The significance level adopted in all of the analyses was of 5% (α =

0.05) and was analyzed using the IBM SPSS Statistics for Windows (IBM SPSS. 21.0, 2012, Armonk, NY: IBM Corp., USA).

## Results

### Sample characterization

The variables related to the demographic factors of the participants from the present study can be observed in Table 1.

The trained and calibrated examiners were categorized according to their exam responses and a Kappa index (K) was calculated to analyze the agreement between them in relation to the result of each parameter and each datum. The intra- and inter-observer agreement obtained ranged from good to excellent (CGk= 1.0 and TGk=0.79).

### Perimplantar and periodontal conditions

The result of the PD, WKM, TKM, and VCF measurements in the implants and the HT are described in Table 2. The PD of the dental implants was significantly higher than that of the HT. By contrast, the WKM of the dental implants was significantly lower than that of the HT. The TKM and VCF presented no statistically significant differences between the dental implants and the HT. The evaluation of the MMP-8 concentration in the perimplantar and periodontal crevicular fluid revealed a statistically significant difference between implants and HT (Fig. 1), given that the MMP-8 expression in the implants was approximately 29% higher than that observed in the HT. The coefficients of correlation between the MMP-8 and the PD, WKM, TKM, and VCF are described in Table 3. A negative correlation between the MMP-8 and the VCF could be observed in the implants and in the HT. No correlation could be observed between the MMP-8 and the other evaluated parameters. No statistically significant difference could be observed between the implants and the HT in the distribution rate of WKM and TKM in specific categories (Table 4). In addition, the MMP-8 concentration in the perimplantar and periodontal crevicular fluids also showed no significant differences in the specific categories of WKM and TKM (table 5).

### Comparison between the different dental implant platforms

The result of the PD, WKM, TKM, and VCF measurements in the implants are described in Table 6. No statistically significant differences regarding the evaluated clinical parameters could be observed between the two types of dental implant platforms. The evaluation of the MMP-8 concentration in the perimplantar fluid of the EH and IH showed no statistically significant differences in the MMP-8 expression between the different platforms (Fig. 2). The correlation coefficients between the MMP-8 and the PD, WKM, TKM, and VCF of the EH and IH are described in Table 7. A negative correlation between the MMP-8 concentration and the VCF could be observed in both the EH and the IH. No correlation could be observed between the MMP-8 expression and the other clinical parameters. No statistically significant difference could be observed in the distribution rates of WKM and TKM in specific categories when comparing the different implant platforms (Table 8). In addition, the MMP-8 concentration in the perimplantar fluid of the EH and IH also differed little in the specific categories of WKM and TKM (Table 9).

## Discussion

The sites of the present study presented no perimplantar disease or periodontitis. The significant differences detected in this study were restricted to a greater PD and a smaller WKM in implants when compared to the HT. It is important to note that these, due to the values found and the information reported and described herein, have not shown clinical impact. The greater MMP-8 expression in the dental implants proved not to be associated with the evaluated parameters, and the VCF presented the same pattern in the comparisons made in this study.

The individuals of the present study presented a predominance of female patients. seeking dental services. aligns with the literature. The larger number of EH implants in the present sample contrasts with prior studies that demonstrate a preference for IH implants due to a perception of less bacterial contamination around the platforms, which could in turn reduce the occurrence of bone loss (Weng et al., 2011; De la Rosa et al., 2013).

In the present study, the PD of the implants was statistically higher than that of the HT, while the WKM was statistically lower than that of the HT (Table 2). This outcome may well be due to the fragility of the perimplantar tissue, as reported by Machtei et al. (2006) and Parpaiola et al. (2014). Moreover, the question of whether

or not the WKM is in fact necessary for the maintenance of perimplantar and periodontal health is controversial, bearing in mind that prior studies have reported that 2mm or more of WKM is important for maintaining these tissues in good health (Schrott et al., 2009; Lang & Löe, 1972; Kim et al., 2009; Bouri et al., 2008; Chung et al., 2006). However, the absence of a sufficient quantity of WKM is not directly related to the occurrence of greater bone loss (Chung et al., 2006; Askin et al., 2013). To obtain the WKM, a periodontal caliper®, was used as previously described by Yared et al. (2006). The WKM of the implants and the HT showed no statistically significant difference (Table 2). This result differs from Chang & Wennström, (2013), who reported a greater gingival thickness in implants. This difference may partly be the result of the manner in which the measurement was taken, as well as of the smaller size of the sample in the previous study.

Differences in the volume of the PICF of EH and IH implants and of the GCF due to the presence of different structures present in these tissues have been described in prior literature (Lang & Berglundh, 2011; Xu et al., 2008), as has the fact that the volumes found correlate with the perimplantar and gingival inflammation (Schierano et al., 2008). No statistically significant differences could be observed in the VCF of the implants and the HT (Table 2). This finding may have resulted from the criteria adopted in the present study, which determined that implants and HT should be clinically healthy.

The evaluation of the MMP-8 concentration in the perimplantar and periodontal crevicular fluids revealed that the expression of this enzyme was significantly higher in the implants (Fig. 1). Prior studies have also reported an increased MMP-8 expression in the perimplantar sites, and even higher levels when perimplantitis was identified (Sorsa et al., 1999; Xu et al., 2008; Chen et al., 1999; Teronen et al., 1997). The possible correlation between the evaluated parameters (PD, WKM, and TKM) and the MMP-8 expression could not be observed in either the implants or the HT. In contrast, a significant negative correlation ($p<0.001$), that is, a greater MMP-8 expression in lower levels of VCF in implants and HT, could be identified (Table 3). Once again, the absence of the correlations between the PD, WKM, and TKM, as well as the similarity with VCF, may well be the result of the heathy clinical condition present in the evaluated implants and HT, which is in accordance with prior studies in the literature (Schrott et al., 2009; Wennstrom et al., 1994; Zitzmann et al., 2001; Salvi & Lang, 2004). In this context, an increased

production of perimplantar and periodontal VCF due to the change in the mucosa, was correlated with previous clinical parameters (Chaytor et al., 1991). In addition, the evaluation of the distribution rate of the WKM and TKM in specific categories, as well as the MMP-8 expression in these categories, showed no statistically significant differences between the implants and the HT (Table 4; Table 5).

The present study also investigated the evaluation of possible differences resulting from the presence of the different implant platforms. No statistically significant differences could be identified between the two different types of implant platforms and the clinical parameters evaluated (PD, WKM, and TKM; Table 6), as well as the MMP-8 expression (Fig. 2). Nevertheless, a significant negative correlation could be observed between the MMP-8 expression and the VCF in EH ($p<0.001$) and IH ($p=0.017$) implants (Table 7). Finally, the evaluation of the distribution rate of the WKM and TKM in specific categories, as well as the MMP-8 expression in these categories, showed no statistically significant differences between EH and IH (Table 8; Table 9). Prior studies also reported similar values in the clinical parameters of the cone morse and IH implants (Zitzmann et al., 2001; Camacho et al., 2012; Dierens et al., 2012), but no studies correlating the clinical parameters and the MMP-8 expression in the different types of EH and IH implants could be found.

## Conclusion

The present study is the first to evaluate the possible influence of different dental implant platforms on an MMP-8. Within the limits of this study, it can be concluded that implants and clinically healthy HT, as well as implants with different platforms, present similar clinical parameters. Additional studies are warranted to improve the comprehension of the origin of the differential MMP-8 expression of implants when compared to HT.

## References

Askin, S.B., Berker, E., Akincibay, H., Uysal, S., Erman, B. & Tezcan, I. (2013) Necessity of keratinized tissues for dental implants: a clinical, immunological, and radiographic study. *Clinical Implant Dentistry and Related Research* (online).

Booth, V. & Lehner, T. (1997) Characterization of the Porphyromonas gingivalis antigen recognized by a monoclonal antibody which prevents colonization by the organism. *Journal of Periodontal Research* **32:** 54-56.

Bouri, A., Bissada, N., Al-Zahrani, M.S., Faddoul, F. & Nouneh, I. (2008) Width of keratinized gingiva and the health status of the supporting tissues aroun dental implants. *The International Journal of Oral & Maxillofacial Implants* **23** (2): 323-326.

Camacho, F.M.T., Sakamura, C.E., Mera, M.F.M., Esteves, J.C., Ribeiro, F.S. & Pontes, A.E.F. (2012) Avaliação clínica em curto prazo do sistema de Cone Morse e plataforma reduzida em prótese sobre implantes do tipo protocolo: estudo piloto randomizado controlado. *Revista de Odontologia da UNESP* **41:** 247-253.

Chang, M. & Wennström, J.L. (2013) Soft tissue topography and dimensions lateral to single implant-supported restorations: a cross-sectional study. *Clinical Oral Implants Research* **24:** 556-562.

Chaytor, D.V., Zarb, G.A., Schmitt, A. & Lewis, D.W. (1991) The longitudinal effectiveness of osseointegrated dental implants. The Toronto Study: bone level changes. *The International Journal of Periodontics and Restorative Dentistry* **11:** 112-125.

Chen, H-Y., Cox, S.W., Eley, B.M., Mäntylä, P., Rönkä, H. & Sorsa, T. (1999) Matrix metalloproteinase-8, elastase, cathepsin B, α2-macroglobulin levels in gingival crevicular fluid from chronic adult periodontitis patients. *Submitted for publication.*

Chung, D.M., Oh, T.J., Shotwell, J.L., Misch, C.E. & Wang, H.L. (2006) Significance of keratinized mucosa in maintenance of dental implants with different surfaces. *Journal of Periodontology* **77:** 1410-1420.

De la Rosa, M., Rodríguez, A., Sierra, K., Mendoza, G. & Chambrone, L. (2013) Predictors of perimplantar bone loss during long-term maintenance of patients treated with 10-mm implants and single crown restorations. *The International Journal of Oral & Maxillofacial Implants* **28:** 798-802.

Dierens, M., Vandeweghe, S., Kisch, J., Nilner, K. & De Bruyn, H. (2012) Long-term follow-up of turned single implants placed in periodontally healthy patients after 16-22 years: radiographic and perimplantarar outcome. *Clin Oral Implants Research* **23:** 197-204.

Graber, H.G., Wilharm, J. & Conrads, G. (1999) Monoclonal antibodies against integrin subunits α6 ant β1 inhibit migration of gingival epithelium in organ culture. *Journal of Periodontology* **70:** 388-393.

Heitz-Mayfield, L.J.A. (2008) Perimplantar diseases: diagnosis and risk indi-cators. *Journal of Clinical Periodontology* **35:** 292-304.

Kim, B.S., Kim, Y.A., Yun, P.Y., Yi, Y.J., Lee, H.J. & Kim, S.G. (2009) Evaluation of periimplant tissue response according to the presence of keratinized mucosa. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **107** (3): 24-28.

Kinane, D.F. (2000) Regulators of tissue destruction and homeostasis as diagnostic aids in periodontology. *Periodontology 2000* **24:** 215-225.

Klokkevold, P.R. & Newman, M.G. (2000) Current status of dental implants: a periodontal perspective. *The International Journal of Oral & Maxillofacial Implants* **15:** 56-65.

Lang, N.P. & Berglundh, T. (2011) Working group 4 of Seventh European workshop on periodontology. Perimplantar diseases: where are we now? Consensus of the Seventh European workshop on periodontology. *Journal of Clinical Periodontology* **38** (Suppl 11): 178-181.

Lang, N.P. & Löe, H. (1972) The relationship between the width of keratinized gingiva and gingival health. *Journal of Periodontology* **43:** 623-627.

Machtei, E.E., Oved-Peleg, E. & Peled, M. (2006) Comparison of clinical, radiographic and immunological parametes. *Clinical Oral Implants Research* **17** (6): 658-665.

Mombelli, A. (1997) Etiology, diagnosis, and treatment considerations in perimplantitis. *Current Opinion in Periodontology* **4:** 127-136.

Parpaiola, A., Cecchinato, D., Taoia, M., Bressan, E., Speroni, S. & Lindhe, J. (2014) Dimensions of the healthy gingiva and perimplantar mucosa. *Clinical Oral Implants Research* (online): 1-6.

Salvi, G.E. & Lang, N.P. (2004) Diagnostic parameters for monitoring perimplantar conditions. *The International Journal of Oral & Maxillofacial Implants* **19** (Suppl): 116-127.

Schierano, G., Pejrone, G., Brusco, P., Trombetta, A., Martinasso, G. & Preti, G. (2008) TNF-a TGF-b2 and IL-1b levels in gingival and perimplantar crevicular fluid before and after de novo plaque accumulation. Journal of Clinical Periodontology **35:** 532-538.

Schrott, A.R., Jimenez, M., Hwang, J.W., Fiorellini, J. & Weber, H.P. (2009) Five-year evaluation of the influence of keratinized mucosa on perimplantar soft- tissue health and stability around implants supporting full-arch mandibular fixed prostheses. *Clinical Oral Implants Research* **20** (10): 1170-1177.

Sorsa, T., Mäntylä, P., Rönkä, H., Kallio, P., Kallis, G.B. & Lundqvist, C. (1999) Scientific Basis of a Matrix Metalloproteinase-8 Specific Chair-side Test for Monitoring Periodontal and Perimplantar Health and Disease. *Annals of the New York Academy of Sciences* **878:** 130-140**.**

Sorsa, T., Tjaderhane, L. & Salo, T. (2004) Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis*eases **10:** 311-318.

Taba Júnior, M., Kinney, J., Kim, A.S. & Giannobile, W.V. (2005) Diagnostic biomarkers for oral and periodontal diseases. *Dental Clinics of North America* **49:** 551-571.

Teronen, O., Konttinen, Y.T., Lindquist, C., Salo, T., Ingman, T. & Lauhio, A. (1997) Human neutrophil collagenase MMP-8 in perimplantar sulcus fluid and its inhibition by clodronate. *Journal of Dental Research* **76:** 1477-1485.

Weng, D., Nagata, M.J.H., Bosco, A.F. & Melo, L.G.M. (2011) Influence of microgap location and configuration on radiographic bone loss around submerged implants: na experimental study in dogs. *The International Journal of Oral & Maxillofacial Implants* **26:** 941-946.

Wennstrom, J.L., Bengazi, F.M. & Lekholm, U. (1994) The influence of the masticatory mucosa on the per-implant soft tissue cognition *Clinical Oral Implants Research* **5** (1): 1-8.

Xu, L., Yu, Z., Lee, H.M., Wolff, M.S., Golub, L.M. & Sorsa, T. (2008) Characteristics of collagenase-2 from gingival crevicular fluid and perimplantar sulcular fluid in periodontitis and perimpalntitis patients: pilot study. *Acta Odontologica Scandinavica* **66** (4): 219-224.

Yared, K.F., Zenobio, E.G. & Pacheco, W. (2006) Periodontal status of mandibular central incisors after orthodontic proclination in adults. *American Journal of Orthodontics and Dentofacial Orthopedics* **130:** 1-8.

Zitzmann, N.U., Scharer, P. & Marinello, C.P. (2001) Long-term results of implants treated with guided bone regeneration: A 5-year prospective study. *The International Journal of Oral & Maxillofacial Implants* **16** (3): 355-366.

## List of Tables

**Table 1. Sample Characterization**

| Parameter | Observations |
|---|---|
| Participants | 47 |
| Age, average ± SD | 49.9 ± 13.8 |
| Gender | Male 14 (29.8%)/Female 33 (70.2%) |
| Dental Implants/homologous teeth | 79/79 |
| Dental implant platform | EH 48 (60.8%)/IH 31 (39.2%) |

SD – standard deviation; EH: external hexagon implant; IH: internal hexagon implant.

**Table 2. Perimplantar and periodontal clinical parameters**

| Parameter | Dental Implants | Homologous Teeth | p |
|---|---|---|---|
| PD (mm)* | 2.00 ± 1.50 | 1.50 ± 1.00 | < 0.001† |
| WKM (mm)* | 2.00 ± 3.00 | 3.00 ± 3.00 | < 0.001† |
| TKM (mm)** | 0.88 ± 0.33 | 0.89 ± 0.30 | 0.817‡ |
| VCF (µL)* | 0.26 ± 0.24 | 0.25 ± 0.00 | 0.079† |

PD – probing depth; WKM – width of keratinized mucosa; TKM – thickness of keratinized mucosa; VCF – volume of crevicular fluid.
* Values expressed in median ± IIQ; ** Values expressed in average ± SD; † Wilcoxon test; ‡ Paired sample Student t test.

**Table 3. Correlation between MMP-8 expression and clinical parameters**

| Parameter | MMP-8 (Dental Implants) | MMP-8 (Homologous Teeth) |
|---|---|---|
| PD | 0.05 (p = 0.700) | - 0.04 (p = 0.741) |
| WKM | - 0.16 (p = 0.232) | 0.17 (p = 0.199) |
| TKM | - 0.05 (p = 0.733) | 0.01 (p = 0.968) |
| VCF | - 0.59 (p < 0.001) | - 0.71 (p < 0.001) |

Spearman rank correlation coefficient; MMP-8: Matrix Metalloproteinase-8; PD – probing depth; WKM – width of keratinized mucosa; TKM – thickness of keratinized mucosa; VCF – volume of crevicular fluid.

**Table 4. Distribution of WKM and TKM in dental implants and homologous teeth.**

| Parameter | Dental Implants | Homologous Teeth | p * |
|---|---|---|---|
| WKM | | | |
| < 2 mm | 33 (41.8%) | 24 (30.4%) | 0.078 |
| ≥ 2 mm | 46 (58.2%) | 55 (69.6%) | |
| TKM | | | |
| < 1 mm | 52 (65.8%) | 53 (67.1%) | 1.000 |
| ≥ 1 mm | 27 (34.2%) | 26 (32.9%) | |

WKM – width of keratinized mucosa; TKM – thickness of keratinized mucosa.
* McNemar test.

**Table 5. MMP-8 concentration in specific categories of WKM and TKM**

| Parameter | Dental Implants | p-value* | Homologous Teeth | p* |
|---|---|---|---|---|
| WKM | | | | |
| < 2 mm | 15515 ± 23971 | 0.466 | 9725 ± 6892 | 0.256 |
| ≥ 2 mm | 14268 ± 24924 | | 12637 ± 9495 | |
| TKM | | | | |
| < 1 mm | 14294 ± 24228 | 0.674 | 9725 ± 8594 | 0.381 |
| ≥ 1 mm | 13796 ± 29291 | | 12754 ± 13968 | |

MMP-8: Matrix Metalloproteinase-8; Median ± IIQ; WKM – width of keratinized mucosa; TKM – thickness of keratinized mucosa.

* Mann-Whitney U test.

**Table 6. Perimplantar clinical parameters**

| Parameter | EH | IH | p* |
|---|---|---|---|
| PD (mm) | 2.00 ± 0.50 | 2.00 ± 1.50 | 0.939 |
| WKM (mm) | 2.00 ± 3.00 | 2.00 ± 3.00 | 0.918 |
| TKM (mm) | 0.86 ± 0.33 | 0.88 ± 0.52 | 0.721 |
| VCF (µL) | 0.26 ± 0.33 | 0.30 ± 0.23 | 0.965 |

IIQ: Interquartile range; EH: external hexagon implant; IH: internal hexagon implant; PD – probing depth; WKM – width of keratinized mucosa; TKM – thickness of keratinized mucosa; VCF – volume of crevicular fluid.
* Mann-Whitney U test.

**Table 7. Correlation between the MMP-8 expression and the clinical parameters in the different dental implant platforms**

| Parameter | MMP-8 (EH) | MMP-8 (IH) |
|---|---|---|
| PD | 0.08 (p = 0.642) | 0.07 (p = 0.742) |
| WKM | - 0.18 (p = 0.277) | - 0.04 (p = 0.864) |
| TKM | 0.07 (p = 0.674) | - 0.24 (p = 0.280) |
| VCF | - 0.62 (p < 0.001) | - 0.50 (p = 0.017) |

EH: external hexagon implant; IH: internal hexagon implant; MMP-8: Matrix Metalloproteinase–8; PD – probing depth; WKM – width of keratinized mucosa; TKM – thickness of keratinized mucosa; VCF – volume of crevicular fluid.
Spearman rank correlation coefficient.

**Table 8. Distribution of WKM and TKM in dental implants with different platforms**

| Parameter | Type of dental implant | | p* |
|---|---|---|---|
| | EH | IH | |
| WKM | | | |
| < 2 mm | 20 (41.7%) | 13 (41.9%) | 1.000 |
| ≥ 2 mm | 28 (58.3%) | 18 (58.1%) | |
| TKM | | | |
| < 1 mm | 34 (70.8%) | 18 (58.1%) | 0.355 |
| ≥ 1 mm | 14 (29.2%) | 13 (41.9%) | |

EH: external hexagon implant; IH: internal hexagon implant; WKM – width of keratinized mucosa; TKM – thickness of keratinized mucosa.
* Chi-squared test.

**Table 9. MMP-8 concentration in specific categories of WKM and TKM in the different dental implant platforms**

| Parameter | EH Median ± IIQ | p-value* | IH Median ± IIQ | p-value* |
|---|---|---|---|---|
| WKM | | | | |
| < 2 mm | 21176 ± 22790 | 0.571 | 12095 ± 47479 | 1.000 |
| ≥ 2 mm | 13508 ± 35146 | | 14556 ± 8853 | |
| TKM | | | | |
| < 1 mm | 17308 ± 25434 | 0.952 | 14243 ± 10921 | 0.443 |
| ≥ 1 mm | 12723 ± 34679 | | 14870 ± 15493 | |

MMP-8: Matrix Metalloproteinase-8; EH: external hexagon implant; IH: internal hexagon implant; WKM – width of keratinized mucosa; TKM – thickness of keratinized mucosa.
* Mann-Whitney U test.

## LIST OF FIGURES

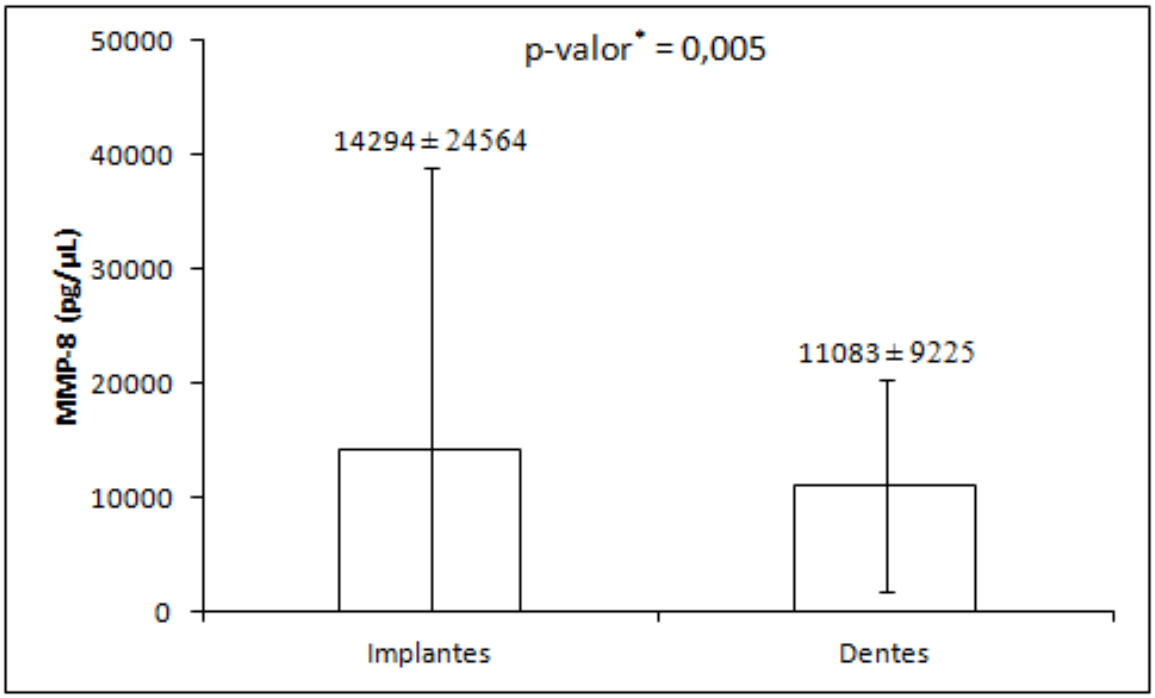


**Fig. 1. MMP-8 concentration in the perimplantar and periodontal crevicular fluid**
MMP-8: Matrix Metalloproteinase–8.
* Wilcoxon test.
Values expressed as median ± IIQ.

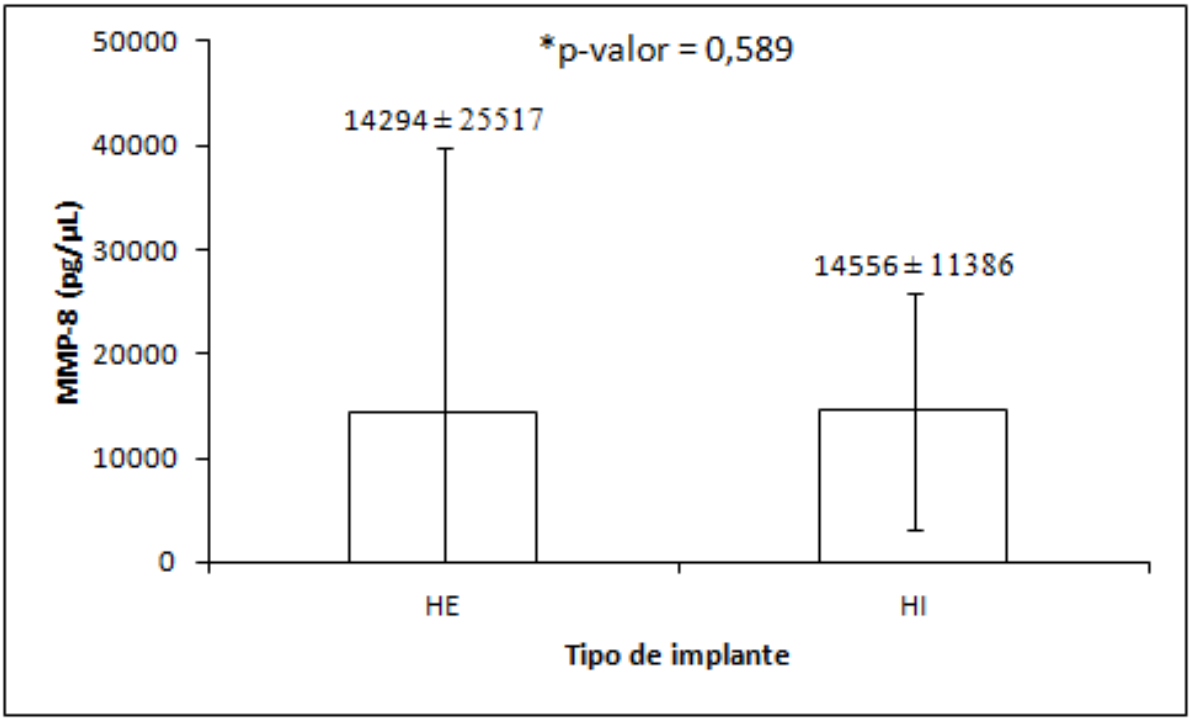


**Fig. 2. MMP-8 concentration in the perimplantar crevicular fluid**

MMP-8: Matrix Metalloproteinase-8; EH: external hexagon implant; IH: internal hexagon implant.
Values expressed as median ± IIQ.
* Wilcoxon test.

## 4 CONSIDERAÇÕES FINAIS

Os resultados deste estudo, na comparação entre implantes e dentes homólogos, mostraram uma maior profundidade de sondagem em implantes e uma maior faixa de mucosa ceratinizada em DH, e que não há diferenças na espessura de mucosa ceratinizada entre os volumes de fluido crevicular. Na comparação entre implantes com conexão externa e conexão interna, não encontramos diferenças entre os parâmetros clínicos avaliados, assim como na expressão da MMP8. Em condições de saúde, implantes e dentes homólogos tem comportamento clínico similar. Novos estudos serão necessários para dimensionar os parâmetros clínicos perimplantares em condições de saúde, mucosite e perimplantite em relação aos periodontais.

## REFERÊNCIAS


BAKER, P.J. The role of immune responses in bone loss during periodontal disease. **Microbes and Infection,** v.10, p. 1181-1192, 2000.

BRANEMARK, P.I. et al. Intraósseus anchorage of dental prosthesis. I . Experimental studies. **Scandinavian Journal of Plastic and Reconstructive Surgery,** v.3, n.2, p. 81-100, 1969.

BRANEMARK, P.I. et al. Branemark novum: a new treatment concept for rehabilitation of the edentulous mandible: preliminary results from a prospective clinical follow-up study. **Clinical Oral Implants Research,** Hamilton, v.1, n.1, p. 2-16, 1999.

BOOTH, V.; LEHNER, T. Characterization of the Porphyromonas gingivalis antigen recognized by a monoclonal antibody which prevents colonization by the organism. **Journal of Periodontal Research,** v.32, p. 54-56, 1997.

GRABER ,H.G.; WILHARM, J.; CONRADS, G. Monoclonal antibodies against integrin subunits α6 ant β1 inhibit migration of gingival epithelium in organ culture. **Journal of Periodontology,** v.70, p. 388-393, 1999.

KINANE, D.F.; LAPPIN, D.F. Clinical, pathological and immunological aspects of periodontal disease. **Acta Odontologica Scandinava,** v.59, p. 154-160, 2001.

MOMBELLI, A. Prevention and Therapy of perimplantar infections. In: LANG, N.P.; KARRING, T.; LINDHE, J. (ed.). **Proceedings of the 3rd European Worshop an Periodontology.** Berlin: Quintessenz Verlag, 1999. p. 281-303.

MONTES, C.C. et al. Analysis of the association of IL1B (Cþ3954T) and IL1RN (intron 2) polymorphisms with dental implant loss in a Brazilian population. **Clinical Oral Implants Research,** v.20, p. 208-217, 2009.

NGUYEN, L. et al. Interleulin-1 Beta Stimulates bone resorption and inhibits bone formation in vivo. **Lymphokine & Cytokine Research,** v.10, n.1-2, p. 15-21, 1991.

RASMUSSEN, L.; HANSTROM, L.; LERNER, U.H. Characterization of bone resorbing activity in gingival crevicular fluid from patients with periodontits. **Journal of Clinical Periodontology,** v.27, n.1, p. 41-52, 2000.

SORSA, T.; TJADERHANE, L.; SALO, T. Matrix metalloproteinases (MMPs) in oral diseases. **Oral Diseases**, v.10, p. 311-318, 2004.

SORSA, T. et al. Scientific basis of a matrix metalloproteinase-8 specific chair-side test for monitoring periodontal and perimplantar health and disease**. Annals of the New York Academy of Sciences,** v.30, n.878, p. 130-140, 1999.

TABA JÚNIOR, M. et al. Diagnostic biomarkers for oral and periodontal diseases. **Dental Clinics of North America,** v.49, p. 551-571, 2005.