HUGO FELIPE DO VALE

PROSPECTIVE, PARALLEL, CONTROLLED TRIAL OF IMPLANTS PLACED IN PATIENTS DIAGNOSED WITH GENERALIZED AGGRESSIVE AND CHRONIC PERIODONTITIS: CLINICAL, MICROBIOLOGICAL, AND IMMUNOLOGICAL EVALUATIONS

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UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

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Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Clinical Dentistry, area of Periodontology

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de doutor em Clínica Odontológica na Área de Periodontia

Supervisor/*Orientador*: Prof. Dr. Márcio Zaffalon Casati

Este exemplar correponde à versão final da tese defendida pelo aluno Hugo Felipe do Vale e orientada pelo Prof. Dr. Márcio Zaffalon Casati

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ABSTRACT

The aim of this study was to evaluate clinical, microbiological, and immunological patterns of dental implants placed in healthy, partially edentulous, patients (HH), and those with a history of aggressive (HGAgP) or chronic periodontitis (HGCP), considering the null hypothesis that there are no differences between groups. This prospective, parallel, controlled trial enrolled 45 patients (HGAgP, n = 13; HGCP, n = 18; HH, n = 14) followed up from implant insertion until six months after implant loading. Plaque index (PI), bleeding on probing (BOP), probing depth (PD), relative clinical attachment level (rCAL), gingival margin position (rGMP), implant stability (IS), and radiographic marginal bone resorption (RMBR) around implants were evaluated. *Porphyromonas gingivalis (Pg)*, *Aggregatibacter actinomycetemcomitans (Aa)*, and *Tannerella forsythia (Tf)* levels were evaluated by real-time PCR. Also, IL-1β, TNFα, IL-6, IL-8, IFNγ, GM-CSF, IL-4, IL-10, RANKL, and OPG levels were evaluated with the LUMINEX/MAGPIX® platform. No inter-group differences were observed around dental implants when the clinical parameters (PI, BOP, PD, rCAL, rGMP, IS, and RMBR) ($p > 0.05$) were considered at any evaluation period. At the first month after implant loading, those in the HGAgP group presented a higher level of *Aa* ($p < 0.05$). Six months after implant loading, those in the HH group presented a lower level of *Pg* ($p < 0.05$). The immunologic evaluation showed higher values of OPG in those in the HH group at implant loading, and a higher IL-4 level in those in the HH group six months after implant loading. It can be concluded that, after six months of implant loading, despite some micro and immunological differences, there are no clinical differences or additional RMBR around implants placed in patients with a history of periodontal disease.

Key-words: aggressive periodontitis, dental implants, bone resorption.

RESUMO

O objetivo deste estudo foi avaliar os parâmetros clínicos, imunoenzimáticos e microbiológicos de implantes dentais de estágio único instalados em pacientes com histórico de periodontite agressiva, periodontite crônica e saúde, considerando a hipótese de nulidade de que não existem diferenças entre os grupos. Foram selecionados pacientes que apresentaram histórico de periodontite agressiva generalizada (PAG) e periodontite crônica generalizada (PCG) com indicação de reabilitação protética implanto suportada. Os pacientes com necessidade de reabilitação unitária foram divididos em 3 grupos: Grupo PAG (n = 13): pacientes apresentando histórico de periodontite agressiva generalizada; Grupo PCG (n = 18): pacientes com histórico de PCG; e Grupo Controle (n = 14): pacientes sem histórico de periodontite. Todos os implantes foram instalados em estágio único e, após 3 meses, receberam reabilitação com próteses metalocerâmicas unitárias parafusadas. Profundidade de sondagem, nível clínico de inserção relativo e posição da margem gengival relativo foram avaliados nos implantes no momento da instalação da prótese e 1, 3 e 6 meses após o carregamento. Avaliação radiográfica foi feita no momento 7 dias após a cirurgia, na instalação da prótese e 6 meses após o carregamento protético. Avaliação microbiológica foi realizada imediatamente após a instalação da prótese, 1, 3 e 6 meses após, por meio de PCR real time, determinando a quantidade dos microrganismos *A. actinomycetemcomitans, P. gingivalis e, T. forsythia* Avaliação imunológica foi realizada utilizando o sistema LUMINEX/MAGPIX® com amostras de fluido periimplantar coletado aos 15 dias após a cirurgia, imediatamente após a instalação da prótese e 6 meses após o carregamento protético, avaliando as concentrações de IL-1β, IL-4, IL-6, IL-8, IL-10, TNFα, INFγ e GM-CSF, além de marcadores de osteogênese e osteoclasia (OPG e RANKL). Não foram observadas diferenças entre os perfis de pacientes quanto aos parâmetros clínicos e radiográficos ao redor dos implantes em nenhum dos períodos de avaliação. No primeiro mês após a instalação das próteses verificou-se maior concentração de *Aa* ($p < 0,05$) no grupo PAG. Seis meses após a instalação das próteses o grupo Controle apresentou menores concentrações de *Pg* ($p < 0,05$). A avaliação dos marcadores de osteogênese/osteoclasia indicou alta concentração de OPG no grupo controle no momento

da instalação da prótese. Ainda neste grupo, alta concentração de IL-4 foi observada 6 meses após o carregamento dos implantes. Dentro das limitações deste estudo, pode ser concluído que 6 meses após a instalação das próteses, não há diferenças clínicas nem adicional reabsorção óssea em implantes dentários instalados em pacientes com histórico de doença periodontal.

Palavras-chave: periodontite agressiva, implantes dentários, reabsorção óssea.

SUMMARY/*SUMÁRIO*

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1 INTRODUCTION/*INTRODUÇÃO*

Oral rehabilitation with dental implants is an important approach for the re-establishment of esthetics and function in partially and totally edentulous patients (Branemark et al., 1977; Buser et al., 1997). Recent systematic reviews have shown good survival and success rates with oral implant rehabilitation (Pjetursson et al., 2004; Ong et al., 2008). However, some studies have demonstrated that patients presenting a history of periodontal disease showed a higher risk of implant failure and greater marginal bone loss than did periodontal healthy individuals (Safii et al., 2010; Donos et al., 2012). Implant survival rates in patients with a history of periodontal disease have ranged from 79.22% to 100% (Hardt et al., 2002; Karoussis et al., 2003; Evian et al., 2004; Roos-Jansaker et al., 2006; Kim & Sung, 2012), while the success rates ranged from 33% to 100% (Karoussis et al., 2003; Mengel & Flores-de-Jacoby, 2005; Swierkot et al., 2012). Most studies have attributed these lower rates to higher susceptibility to peri-implant mucositis and peri-implantitis in patients with a history of periodontal disease (Donos et al., 2012).

The higher risk of peri-implant infection in individuals with periodontitis could be explained by microbiological linkage. Studies have demonstrated the association of *Aggregactibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia*, and *Prevotella intermedia* with peri-implant diseases (Casado et al., 2011; Cortelli et al., 2013). At the same time, it has been demonstrated that, even after tooth loss, key periodontal pathogens remain, colonizing the oral cavity (Fernandes et al., 2010). Thus, adjacent periodontal sites, the tongue, tonsils, and saliva can be reservoirs of pathogenic microorganisms (Quirynen et al., 2005; Aoki et al., 2012; Ito et al., 2013) and can aggressively colonize peri-implant sites. However, the presence of these bacteria *per se* is not a determining factor for disease onset, since pathogens can also be found in healthy peri-implant conditions (Casado et al., 2011; Cortelli et al., 2013). Thus, in addition to the bacterial load around implants, host susceptibility by an altered

inflammatory response could influence the homeostasis of dental implants inserted into patients with periodontitis.

Previous studies have indicated that the peri-implant gingiva may be characterized by a high pro-inflammatory state, even under apparent homeostatic conditions (clinically healthy tissues) (Nowzari et al., 2008). The presence of cytokines like IL-1β, TNFα, and IL-8 can be a predictor of peri-implant destruction (Petkovic et al., 2010). Thus, an analysis of inflammatory factors is important when individuals at higher risk for periodontitis are considered for implant therapy. This becomes even more important in the consideration of aggressive periodontitis, which presents an imbalance between pro- and anti-inflammatory cytokines (Teles et al., 2010) and could explain the most prevalent mucositis and peri-implantitis assessed in this population (Swierkot et al., 2012).

In this context, in view of the few studies in the literature that have investigated this condition, the aim of this study was to evaluate clinical, microbiological, and immunological patterns of dental implants placed in healthy, partially edentulous, patients, and those with a history of aggressive or chronic periodontitis, considering the null hypothesis that there are no differences between groups.

2 LITERATURE REVIEW/*REVISÃO DA LITERATURA*

Survival, success and implant bone loss in patients with history of periodontal disease

Osseointegrated implants are widely used to treat partially and totally edentulous patients. This kind of approach has presented high levels of success and survival rates and has been indicated as a predictable treatment strategy. Despite its predictability, some risk factors can negatively influence the results of implant therapy, leading to implant loss or increasing the occurrence of implant complications, such as periimplant infections.

The history of periodontal disease has been reported as a risk factor for implant dentistry. Recent meta-analysis and systematic reviews have highlighted the hypothesis that patients with history of periodontal disease present higher chance to pursuit implant loss, periimplantitis and implant-bone loss, than healthy patients (Safii et al., 2010; Kim et al., 2012; Sgolastra et al., 2013). Nonetheless, this is not a consensus among all the studies in the literature.

Mengel and Flores-de-Jacoby (2005) developed a prospective longitudinal study following 150 implants placed in 39 patients. Clinical and radiographic comparisons were made between patients treated for generalized aggressive periodontitis (GAP), generalized chronic periodontitis (GCP) and healthy patients. After 3 years, the implant success rates recorded were 100% in the periodontally healthy (PH) and GCP patients, and 95.7% in the maxilla and 100% in the mandible of GAP patients. Probing depth (PD) and attachment level (AL) around implants were 3.17 mm and 4.30 mm in GAP group, 2.69 mm and 4.57 mm in GCP group and 3.28 and 4.03 mm in periodontally healthy patients, without difference between groups. Considering radiographic bone loss (BL), after 3 years GAP, GCP and healthy patients presented 1.14 mm, 0.86 mm and 0.70 mm of BL respectively.

Ferreira et al. (2006) performed a study which aim was to verify the prevalence of peri-implant disease and to analyze possible risk variables associated with peri-implant

diseases. After clinical and radiographic evaluations of 212 partially edentulous subjects, the authors performed a multinomial regression analysis that indicated the presence of periodontitis as one of the variables associated with increased risk of peri-implantitis. In this study the prevalence of peri-implant mucositis and peri-implantitis were 64.6% and 8.9%, respectively.

Mengel et al. (2007) developed another prospective 10-year clinical and radiographic study of partially edentulous subjects treated for GAP (5 subjects) comparing their implants with those of PH patients (5 subjects). The peri-implant PD was similar in the two groups and remained less than 5 mm throughout the follow up. Attachment loss was higher in GAP group (2.4 mm). The implant success rate differed between groups (83.33% for GAP and 100% for PH). The mean of peri-implant BL at 10-year follow up was 3.37 mm for GAP group and 1.24 for PH group. The authors concluded that GAP patients can be successfully rehabilitated with osseointegrated implants, however, it can be expected greater bone and attachment loss at implants placed in these kind of patients.

Gatti et al. (2008) evaluated the outcome of dental implants placed in partially edentulous patients with a history of severe periodontitis (26 patients and 129 implants), moderate periodontitis (7 patients and 26 implants) and no history of periodontitis (29 patients and 72 implants). After 5 years, patients affected by severe and moderate periodontitis lost on average twice the amount of peri-implant bone compared with the healthy patients (2.6 mm versus 1.2 mm). Thus, concluded that patients with history of periodontitis lose more peri-implant bone than PH patients.

De Boever et al. (2009) followed 16 GAP patients, 68 GCP patients and 110 PH patients in a prospective clinical trial during no more than 6 years. In spite of the presence of confounding factors like smoking habits and bone regeneration procedures, the authors observed survival rates of 84.8%, 96% and 97% in GAP, GCP and PH patients, respectively. Also, difference between groups was found at peri-implant bone loss evaluation. They reported that marginal bone loss per year was 0.12 and 0.08 mm in GCP

and PH patients and 0.17 mm in GAP patients. Once more, generalized aggressive periodontitis patients presented lower results of implant therapy.

Koldsland et al. (2009) performed a study to investigate the prevalence of implant loss and the factors associated with the outcome of dental implants. With an average of 8.4 years (range, 1.1 to 16 years) after oral rehabilitation using dental implants, 109 patients (372 implants) were clinically and radiographically evaluated. Eighteen implants (4.8%) were lost in 10 subjects. Eleven implants were lost before loading, three were lost during the first 5 years after loading, and four were lost 5 to 10 years after loading. The loss of implants was significantly associated with a history of smoking and periodontitis ($p < 0.05$) and the survival rate at subject and implant level were 90.8% and 95.2%.

Roccuzzo et al. (2010) aimed to compare the long-term outcomes of implants placed in periodontally compromised patients and in PH patients. At 10 years after oral rehabilitation with dental implants, clinical measurements and radiographic bone changes were recorded. During the period of observation, 18 implants were removed because of biological complications. The implant survival rate was 96.6% (PH patients), 92.8% (moderate periodontitis patients) and 90% (severe periodontitis patients). The mean bone loss was 0.75 mm in PH patients, 1.14 mm in moderate periodontitis patients, and 0.98 mm in severe periodontitis patients, but without difference between groups. Patients with severe periodontitis experimented more sites presenting bone loss ≥ 3mm than PH patients. The authors concluded that patients with history of periodontitis presented a lower survival rate and a higher number of sites with peri-implant bone loss.

Levin et al. (2011) aimed to evaluate the long-term survival rate of dental implants according to the patient's periodontal status. The prospective study evaluated 736 patients and 2336 dental implants during an average of 54.4 months. The cumulative survival rates at 108 months were 96%, 95% and 88% for, healthy, moderate chronic and severe chronic periodontal patients, respectively. Severe chronic disease turned out to be a significant risk factor for implant failure after 50 months of follow up (Hazard ratio = 8.06;

$p < 0.01$). Thus, periodontal status was considered a significant risk factor for late implant failure.

Swierkot et al. (2012) developed a prospective study following patients during 5 to 16 years to investigate the prevalence of mucositis, peri-implantitis, implant success and implant survival rates in partially edentulous subjects. Thirty-five GAP patients and 18 PH patients were rehabilitated with osseointegrated implants and presented implant survival rate of 100% in PH patients and 96% in GAP patients. In spite of that, the implant success rate was 33% in GAP and 50% in PH group. In GAP patients, mucositis was presented in 56% and peri-implantitis in 26% of the implants. In periodontally healthy individuals, 40% of the implants showed mucositis and 10% peri-implantitis. So, GAgP patients had a five times greater risk of implant failure, a three times greater risk of mucositis, and a 14 times greater risk of peri-implantitis.

Jiang et al. (2013) evaluated the efficacy of implant supported dental restorations in 30 patients with (149 implants) and 30 patients without (127 implants) periodontal disease within 2 years of completing the treatment. There was no difference in success rate between groups (95.97% for periodontitis group and 97.60% for healthy group). In spite of that, modified plaque index and modified sulcus bleeding index were positively correlated with chronic periodontal disease.

Casado et al. (2013) developed a study in Brazil aiming to assess if patients with history of chronic periodontitis were more susceptible to peri-implant disease then those without history of periodontitis. The authors evaluated 215 patients with 754 dental implants. Patients were divided into 2 groups according to the peri-implant status: control group (129 patients without peri-implant disease) and test group (86 patients with peri-implant disorders). There was a highly significant correlation between chronic periodontitis history and peri-implant disease ($p < 0.0001$). Patients with periodontitis had 4 times more chance of developing peri-implant diseases than patients with healthy periodontal tissues. Also, periodontitis patients showed higher bone loss around implant ($p = 0.004$) when compared with patients without periodontitis.

Marrone et al. (2013) evaluated a Belgian population to verify the frequency of mucositis and peri-implantitis in patients with dental implants with at least 5 years of function. One hundred and three patients (38 males/65 females) with a total of 266 implants were examined and the mean time of implants in function was 8.5 years (±3.2). Prevalences of mucositis and peri-implantitis at patient's level were respectively 31% and 37%. They were 38% and 23% at the implant's level. Subjects with active periodontitis (OR = 1.98) were found to be prone to peri-implantitis. Consequently, patients with such characteristic should be informed before implant placement and frequently re-called for maintenance visits.

Roccuzzo et al. (2013) evaluated, clinically and radiographically, 123 partially edentulous patients 10 years after oral rehabilitation with dental implants. No difference between periodontitis and healthy groups occurred in implant survival rate. The percentage of implants with at least one site presenting probing depth > 5 mm was higher in periodontitis patients (9.4% in moderate and 10.8% in severe periodontitis patients) than in healthy patients (0%).

Trying to explain the issue about crestal bone changes around teeth and implants, Rasperini et al. (2013) developed a 10-year radiographic evaluation of 120 oral rehabilitations (60 patients with history of periodontal disease and 60 PH patients). At 10 years post therapy, the survival rate ranged from 80% to 95% for subgroups for implants in both groups. Greater bone loss was observed in smoker patients with history of periodontal disease (average 3.01 mm).

Continuing the marginal bone loss investigation, Galindo-Moreno et al. (2014) analyzed marginal bone loss rates around implants. Five hundred and eight implants were placed in 208 patients. Data were gathered on age, gender, bone stratum, prosthetic connection, smoke and alcohol habits, and previous periodontitis. The results showed that most of the implants with higher bone loss were found in a low portion of the patients, similar to the pattern observed for periodontitis. Nonetheless, a history of periodontitis was not significantly related to the marginal bone loss in this study.

In spite of this controversial results showed in the literature, recent systematic reviews and meta-analysis have pointed the history of periodontal disease as risk factor for dental implant therapy. Safii et al. (2010) purposed to evaluate the risk for marginal bone loss around implants and implant failure in subjects with a history of periodontitis compared with periodontally healthy subjects in studies with a minimum 3-year follow-up. The odds ratio for implant survival was significantly in favor of PH patients (3.02, 95% CI 1.12-8.15). A random effects model showed more marginal bone loss in periodontitis subjects compared with periodontally healthy subjects (standard mean difference 0.61, 95% CI 0.14-1.09). So, a moderate level of evidence indicates that periodontitis subjects were at significantly higher risk for implant failure and greater marginal bone loss than PH subjects.

With the purpose of analyzing the current literature and to assess outcomes of implant treatment in GAP patients, Kim et al. (2012) developed a systematic review divided the studies in short term (< 5 years) and long term studies (≥ 5 years). Seven prospective studies were selected, including four short-term and three long-term studies. The survival rates of the superstructures were generally high in patients with GAP, i.e. 95.9 - 100%. Marginal bone loss around implant in patients with GAP as compared to implants in patients with chronic periodontitis or periodontally healthy patients was not significantly greater in short term studies but was significantly greater in long-term studies. In short term studies, the survival rates of implants were between 97.4% and 100% in patients with GAP-associated tooth loss, except in one study. The survival rates of implants were between 83.3% and 96% in patients with GAP in long-term studies.

Sgolastra et al. (2013) developed a systematic review and meta-analysis to assess the role of periodontal disease as a risk factor for implant loss, peri-implantitis and implant bone loss. Meta-analysis revealed that a higher and significant risk for implant loss was present in patients affected by periodontal disease (RR: 1.89, 95% CI: 1.35 – 2.66, p = 0.0002), but the risk was increased for patients with aggressive periodontitis (RR: 4.04, 95% CI: 1.81 – 8.98, p = 0.0006). A higher and significant implant bone loss was presented in patients with periodontal disease, when compared with PH patients (0.44 mm, 95% CI:

0.19 – 0.69, p = 0.0006). Periodontally compromised patients showed an increased risk for peri-implantitis, when compared with PH patients (RR: 2.21, 95% CI: 1.42 – 3.43 p = 0.0004). In conclusion, there is a strong evidence suggesting periodontitis as a risk factor for implant loss, moderate evidence revealed that periodontitis is a risk factor for peri-implantitis and that patients with periodontitis have higher implant bone loss.

Finally, Faggion & Giannakopoulos (2013) critically revised the systematic reviews that evaluated the effect of a history of periodontitis on dental implant loss. The authors concluded that the methodological quality of the sample of systematic reviews was extremely heterogeneous. In spite of that, most systematic reviews report a positive association between history of periodontitis and risk of implant failure.

Microbiological aspects of implants

The studies have shown that peri-implant diseases have common characteristics with periodontal diseases. These are: inflammation process involving biofilm and host susceptibility. So the presence of specific bacteria could influence the outcomes of implant rehabilitation. Since bacteria colonization begins immediately after implant exposure in oral environment, saliva, tonsils, mucosa, tongue and persistent periodontal pockets are considered sources for peri-implant sulcus. Therefore, patients with history of periodontitis could have pathogenic bacteria colonizing this structure.

Apse et al. (1989) investigated the soft tissues adjacent to osseointegrated implants using clinical, biochemical and microbiological methods. The subgingival bacterial flora was examined and cultured. Few differences were observed between implants and teeth in partially edentulous patients, indicating that crevices around teeth may act as reservoirs of bacteria, which can colonize implant sites. A higher percentage of black-pigmented bacteria and wet spreaders (*Capnocytophaga*) was noticed at partially edentulous implant sites when compared with edentulous implant sites, perhaps, reflecting the lower number of periodontal pathogens present in edentulous mouths. Overall, the

characteristics of implant sulci appear to be similar to periodontal sulci with respect of crevicular fluid flow and microflora.

Quirynen & Listgarten (1990) evaluated the subgingival plaque around implants by means of differential phase-contrast microscopy. In 24 partially edentulous patients (with implants and teeth in the same jaw), no significant difference in the distribution of bacterial morphotypes could be found between implants and natural teeth. However, when the plaque composition on the implants of fully edentulous patients was compared with those of teeth or implants of partially edentulous patients (with teeth and implants in the same and/or opposite jaw), significant differences appeared. In fully edentulous patients, more coccoid cells (71.3%) and significant fewer motile rods (0.4%) and spirochetes (0.0%) were found around the implants. The results suggest that teeth may serve as a reservoir for the bacterial colonization of titanium implants in the same mouth.

Mombelli et al. (1995) evaluated subgingival microbial samples from deepest residual pockets of patients with a history of periodontitis before the implant insertion, and samples of peri-implant sulcus in the same patients 3 and 6 months after implant insertion. The samples were cultured using continuous anaerobic techniques. The authors found presence of *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium* and spirochetes around implants in those patients that previously presented these kinds of bacteria in the residual pockets. So it suggested that bacterial colonization could occur from residual pockets to peri-implant sulcus.

Papaioannou et al. (1996) examined the relation between the subgingival flora around implants and their periodontal parameters in 279 patients (561 implants). The impact of the intraoral exposure time on the microbial composition around the implants was cross-sectionally examined, with the same group of patients, but only tendencies could be detected by the latter, and no concrete conclusions could be drawn. For partially edentulous patients, there was a tendency for increased proportions of spirochetes and motile organisms the longer the intraoral exposure time. These observations emphasize the importance of the periodontal health of the remaining teeth (as a reservoir of pathogenic

microorganisms) in partial edentulous patients rehabilitated by means of implants and indicate the importance of shallow pockets around implants.

Papaiouanou et al. (1996) examined, in partially edentulous patients with severe periodontitis, whether pockets around teeth and implants harbored a comparable micro-flora. In 6 patients (3 with refractory periodontitis and 3 with advanced chronic adult periodontitis), plaque samples were taken from a deep and shallow pocket around both teeth and implants for differential phase contrast microscopy and DNA probe analysis. The results showed important differences in the subgingival flora between the 2 disease groups, as well as between deep and shallow pockets, around both implants and teeth. On the other hand, when pockets around teeth and implants with equal depths were compared a striking similarity was observed in the microbial composition. These observations confirm the hypothesis that pockets around teeth act as a reservoir and highlight the importance of periodontal health when oral implants are planned.

Quyrinen et al. (1996) found that the subgingival flora around the implants harbored more spirochetes and motile rods when teeth were present in the same jaw ($p < 0.05$) and/or when the pockets around them harbored a pathogenic flora ($p < 0.05$). They also investigated the impact of periodontitis around the remaining teeth and the impact of probing depth around the implants on the composition of the peri-implant subgingival flora. The samples from deep pockets (≥ 4 mm) around implants showed significant increase in the total proportion of spirochetes and motile organisms when compared to samples from healthy subjects (1.2%) or in chronic periodontitis patients (21.0%), or in patients suffering from refractory periodontitis (31.5%). The findings of this study confirmed the transmission of microorganisms from teeth to implants.

Gouvoussis et al. (1997) investigated 25 tooth and implant sites for the presence of putative periodontopathic organisms, using specific DNA probes for *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Eikenella corrodens, Fusobacterium nucleatum, Treponema denticola,* and *Campylobacter recta*. Five of nine patients showed a likelihood of transmission from tooth to implant sites. These

patients also showed a high number of putative periodontopathic organisms present in the tested tooth sites. A significant risk was found for transmitting putative periodontopathic organisms from periodontitis sites to implant sites in the same mouth.

Lee et al. (1999) evaluated implant colonization by species in a newly described red complex of periodontal pathogens, *Porphyromonas gingivalis* and *Bacteroides forsythus*. Forty-three partially edentulous subjects with successfully osseointegrated dental implants were examined and the microbiota from peri-implant sites were analyzed using DNA probes in a checkerboard assay. Implants were mainly colonized by oral *streptococci, capnocytophagae, Veillonella parvula, Peptostreptococcus micros,* and *Fusobacterium nucleatum*. The periodontal species, *P. gingivalis, B. forsythus, Prevotella intermedia, Prevotella nigrescens,* and *Campylobacter rectus* were detected in few subjects. Microbial complexity increased as loading time increased, but colonization by periodontal pathogens, including red complex species, was higher in subjects with previous periodontal disease.

Sbordone et al. (1999) purposed to determine the clinical status and the composition of the subgingival microbiota of dental implants and natural teeth in patients with a history of periodontitis. The authors evaluated 42 implants, in 25 subjects with history of moderate periodontal disease, during 3 years. Probing depth and clinical attachment level measurements of peri-implant sites did not show any statistically significant difference. There was no difference detected in the subgingival microbiota, culturally identified at peri-implant and periodontal sites for the duration of the study. In conclusion, implants were colonized by the indigenous periodontal microbiota and were well maintained in patients with a history of periodontitis. No significant association between progressing or non-progressing periodontal or peri-implant sampled sites in terms of loss of attachment and infection with at least one of the searched periodontal pathogens was found, suggesting that the presence of putative periodontopathogens at peri-implant and periodontal sites may not be associated with future attachment loss or implant failure.

Sumida et al. (2002) examined colonization by periodontopathic bacteria and their transmission from periodontal pockets to peri-implant sulcus. Samples were collected

from 105 sites in 15 patients and the transmission of periodontopathic bacteria from periodontal sites of natural teeth to the implant sulcus was analyzed by pulsed field gel electrophoresis (PFGE). The PCR detection rates of *Porphyromonas gingivalis, Prevotella intermedia, Actinobacillus actinomycetemcomitans, Bacteroides forsythus,* and *Treponema denticola* were 80.0%, 53.3%, 46.7%, 60.0% and 40.0%, respectively. Colonizations by *P. gingivalis* and *A. actinomycetemcomitans* were statistically correlated with periodontal pockets and implant sulcus regions ($p < .01$). These analyses indicated that there appeared to be transmission of *P. gingivalis* and *P. intermedia* from the periodontal pocket to the peri-implant region.

Quirynen et al. (2005) evaluated the early colonization of 'pristine' pockets created during implant surgery in 16 partially edentulous patients. Four subgingival plaque samples were taken from shallow and medium pockets around implants 1, 2 and 4 weeks after abutment connection. Checkerboard DNA-DNA hybridization and culture data revealed the development of a complex microbiota, including species from red complex, in the 'pristine' pockets within 1 week.

Quirynen et al. (2006) followed the colonization of 'pristine' sulci created in 42 partially edentulous patients during implant surgery (e.g. abutment connection). Per patient, four subgingival plaque samples were taken from shallow and medium pockets around implants (test sites), and teeth within the same quadrant (undisturbed microbiota as control sites), 1, 2, 4, 13, 26 and 78 weeks after abutment connection, respectively. Checkerboard DNA-DNA hybridization and real-time PCR revealed a complex microbiota (including several pathogenic species) in the peri-implant pockets within 2 weeks after abutment connection. After 7 days, the detection frequency for most species (including the bacteria associated with periodontitis) was already nearly identical in samples from the fresh peri-implant pockets (5% and 20% of the microbiota belonging to red and orange complex, respectively) when compared with samples from the reference teeth. Afterwards (between weeks 2 and 13), the number of bacteria in peri-implant pockets only slightly increased (+/- 0.1 log value), with minor changes in the relative proportions of bacteria associated with periodontitis (8% and 33% of the microbiota belonging to red and orange complex,

respectively). Although small differences were seen between teeth and implants at week 2 with cultural techniques, a striking similarity in subgingival microbiota was found with this technique from month 3 on. So, this study indicated that the initial colonization of peri-implant pockets with bacteria associated with periodontitis occurs within 2 weeks.

De Boever & De Boever (2006) evaluated the early colonization of non-submerged implants over a 6-month period in partially edentulous patients treated for advanced aggressive periodontal disease. In 22 patients, 68 non-submerged dental implants were installed. Using DNA-probes, the presence and concentration of five periodontal pathogens (*Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis* and *Treponema denticola*) were determined in the five deepest pockets of the rest dentition pre-operatively and after 6 months as well as five places around each implant 10 days, 1 month, 3 months and 6 months after surgery. In each patient, a test to determine the genotype interleukin-1 (IL-1) was performed. After 6 months, no difference in microbial composition compared with baseline was found around the teeth in five patients. Ten days after surgery, three patients had a complete similar bacterial composition between teeth and implants. In 14 patients, the composition was fairly similar, while large differences in composition and concentration occurred in five patients. This microbiota around the implants remained almost unchanged over a 6-month period and did not hamper the clinical and radiographic osseointegration and did not lead to peri-implantitis, mucositis or initiation of bone destruction.

Mombelli & Décaillet (2011) conducted a literature review and described the microbiota associated with peri-implant disease. In most studies bacterial samples were obtained by methods that destroyed the three-dimensional structure of the biofilm. The samples therefore describe mixtures of bacteria from unspecified districts of biofilm associated with peri-implant diseases. Analyses of such samples with various methods indicate that peri-implant disease maybe viewed as a mixed anaerobic infection. In most cases the composition of the flora is similar to the subgingival flora of chronic periodontitis that is dominated by gram-negative bacteria.

Lachmann et al. (2012) provided a clinical and microbiological investigation in 74 subjects. Signs of a serious peri-implantitis condition were not encountered. However, a high prevalence of moderate plaque and bleeding on probing (60% and 78%) and PCR proof of periodonto-pathogenic bacteria (43% positive for one or more target species) were apparent. The authors pointed that a considerable number of individuals exhibited peri-implant findings that would require anti-infective treatment.

Cortelli et al. (2013) evaluated the presence of *Porphyromonas gingivalis, Tannerella forsythia, Campylobacter rectus, Prevotella intermedia, Treponema denticola* and *Aggregatibacter actinomycetemcomitans* in peri-implant tissues presenting health, mucositis and peri-implantitis. Using DNA Checkboarding the authors worked with the frequency of presence of each bacteria. Except for *P. intermedia*, bacterial frequency was higher in peri-implantitis than in health. The frequency of *P. gingivalis* and red complex species were higher in peri-implantitis than in mucositis.

Dierens et al. (2013) investigated the microbiota around single implants after 16 to 22 years. Samples were analyzed by DNA-DNA hybridization including 40 species. *Tannerella forsythia* showed the highest concentration around implants and *Porphyromonas gingivalis, Prevotella intermedia, Parvimonas micra* and *Treponema denticola* were also found around implants. Total DNA count was correlated to interproximal bleeding index ($r = 0.409$) and interproximal probing depth ($r = 0.307$). No correlations were present with plaque index or radiographic bone level. In spite of the presence of pathogenic bacteria, the majority of implants presented healthy peri-implant tissues without bone loss.

Ito et al. (2013) purposed to investigate whether the periodontal pathogen levels in saliva were correlated with the periodontal status of patients receiving implant treatment. Two hundred and ninety-one patients were divided into four groups: a no-periodontitis (np) group, a mild-periodontitis (mip) group, a moderate-periodontitis (mop) group, and a severe-periodontitis (sp) group. The levels of the following five periodontal pathogens in saliva were evaluated using real-time polymerase chain reaction: *Porphyromonas*

gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Treponema denticola, and *Prevotella intermedia.* The levels of *P. gingivalis* and *T. forsythia* were significantly higher in mop group than in np group ($p < 0.05$). The levels of all periodontal pathogens tested except *A. actinomycetemcomitans* were significantly higher in sp group than in np group ($p < 0.05$). The detection levels of the periodontal pathogens targeted in saliva samples were correlated with the periodontal status.

According to the information collected in the literature, peri-implant environment presents a complex microbiota that can be harbored by known periodontopathic bacteria. In this context patients with history of periodontal disease could present reservoirs of gram-negative bacteria that would colonize the peri-implant sulcus. So, history of periodontal disease should be evaluated cautiously and any type of periodontal disease has to be treated before dental implant insertion. Also, the care with this kind of patient needs to continue after oral rehabilitation with implants to avoid or treat peri-implant diseases.

Immunological aspects of implants

Peri-implant diseases present inflammatory component and alterations in citokines levels occurred in the peri-implant tissues. The early detection of these alterations could help clinicians to avoid or to treat mucositis and peri-implantitis increasing the longevity of oral rehabilitation with dental implants. Therefore, the analysis of peri-implant crevicular fluid (PICF) would offer a non-invasive mean of studying the host response in peri-implant disease and might provide an early indication of patients at risk for active disease.

Petkovic et al. (2010) examined the PICF levels of interleukin-1beta (IL-1b), tumour necrosis factor alpha (TNF-α), interleukin-8 (IL-8) and macrophage inflammatory protein-1alpha (MIP-1α) in patients with non-manifesting inflammation, early and late stages of mucositis. The authors evaluated 90 adult healthy volunteers with dental implants

inserted. Implant tissues were categorized clinically as healthy, early mucositis or advanced mucositis. Patients from the control group (healthy patients) had significantly lower concentrations of IL-1b, TNF- α, IL-8 and MIP-1 α in PICF compared with both groups with mucositis. Positive correlation was noticed in the control group between IL-1b and TNF- α and between MIP-1 α and IL-8 in the group with early mucositis. The results suggest that cytokines could be prognostic markers of implant failure.

Rakic et al. (2012) investigated the levels of biomarkers associated with osteoclastogenesis in patients suffering peri-implantitis and compared them with levels in healthy peri-implant sites and severe chronic periodontitis. Peri-implant/gingival crevicular fluid samples and clinical parameters were collected from 70 patients (23 with peri-implantitis, 25 with healthy peri-implant tissues and 22 with severe chronic periodontitis). The concentrations of sRANKL, RANK and OPG were evaluated using enzyme-linked immunosorbent assays. sRANKL ($p = 0.01$), RANK ($p = 0.01$) and OPG ($p = 0.03$) concentrations were significantly higher in peri-implantitis sites when compared to those in healthy implant sites, although differences in the sRANKL/OPG ratio were not statistically significant. In these sites all three markers were significantly correlated with the clinical parameters, with exception of OPG/plaque index correlation that remained insignificant ($p = 0.121$). When comparing peri-implantitis and periodontitis findings, RANK was significantly higher in peri-implantitis sites whereas, sRANKL ($p = 0.03$) and sRANKL/OPG ratio ($p = 0.004$) were significantly higher in periodontitis sites. Among periodontitis and healthy implant sites the same differences have been observed for both sRANKL ($p = 0.000$) and sRANKL/OPG ratio ($p = 0.000$), furthermore RANK was higher in periodontitis sites as well ($p = 0.010$). In conclusion, these results suggested that the PICF levels of biomarkers sRANKL, RANK, and OPG are associated with peri-implant tissue destruction and the pattern of these biomarkers differed when compared to periodontitis.

Fonseca et al. (2012) aimed to measure the levels of GM-CSF, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IFN-c and TNF-α in peri-implant crevicular fluid (PICF) and saliva from patients with peri-implant disease. In PICF, the levels of IL-1b were

significantly higher in shallow peri-implantitis sites compared to mucositis ($p = 0.03$). In the saliva from parotid, IL-8 and IL-12 were significantly higher in patients with peri-implantitis ($p = 0.04$). The authors concluded that elevated levels of IL-1b in PICF seem to be a characteristic trait of patients with peri-implantitis.

Güncü et al. (2012) aimed to analyze PICF interleukin-1 beta (IL-1b), IL-10, osteoprotegerin (OPG), receptor activator of nuclear factor-kappa B ligand (RANKL) levels to determine whether the diagnostic value of PICF can be used to evaluate early changes around implants. A total of 47 dental implants either healthy/non-inflamed ($n = 20$) (Group I), or gingivitis/inflamed ($n = 27$) (Group II), were classified. Volume of PICF was statistically higher in Group II. IL-1b, IL-10 and OPG levels in PICF were significantly higher in Group II. RANKL levels did not differ between groups. These data suggest that a balance of inflammatory and osteoclastogenesis related molecules locally produced may play an important role in the development of inflammatory peri-implant lesions.

Emecen-huja et al. (2013) evaluated peri-implant (PICF) and gingival crevicular fluids (GCF) at surgery and 12 weeks after implant insertion in 40 subjects. PICF volume decreased threefold by week 12 ($p = 0.0003$). IL-6, IL-8, MIP-1b and TIMP-1 levels significantly increased at surgical sites at week one, significantly decreasing thereafter ($p < 0.016$). Peri-implant gingival healing, as determined by crevicular fluid molecular composition, differed from periodontal healing. The observed differences suggest that peri-implant tissues, compared to periodontal tissues, represent a higher pro-inflammatory state.

Yaghobee et al. (2013) investigated the relationship between the concentration of IL-1_β in gingival crevicular fluid (GCF) and peri-implant crevicular fluid (PICF) and clinical parameters such as plaque index (PI), gingival index (GI), pocket depth (PD) and bone loss (BL). The authors evaluated 32 patients and found positive correlation between the level of IL-1_β and PI, GI, PD and BL in implants and in teeth ($p < 0.0001$). In similar conditions, the level of IL-1_β was greatly higher in PICF than GCF (75.26 pg/µl and 45.71 pg/µl, respectively) ($p = 0.001$). So, the findings of the present study indicated that the level

of IL-1_{β} may be an important supplement to clinical findings in measuring the health status of gingival or peri-implant tissues.

There are few studies in the literature regarding peri-implant cytokines levels. So no consensus could be achieved and more studies should be developed to clarify this issue.

3 PROPOSITION/PROPOSIÇÃO

The aim of this study was to evaluate clinical, microbiological, and immunological patterns of dental implants placed in healthy, partially edentulous, patients (HH), and those with a history of aggressive (HGAgP) or chronic periodontitis (HGCP), considering the null hypothesis that there are no differences between groups.

4 MATERIAL AND METHODS/MATERIAL E MÉTODOS

Study Population and Design

The study protocol and informed consent form were approved by the Ethics Committee of Piracicaba Dental School (number 017/2010).

This prospective, parallel, controlled clinical trial was designed in accordance with the CONSORT Statement (Moher et al., 2010). The study began with 49 partially edentulous patients who had accepted a treatment plan for single-tooth implant-retained rehabilitation. The convenience sample was recruited from the Graduate Periodontology Clinic of Piracicaba Dental School – State University of Campinas, Piracicaba, Brazil. The selection occurred between March 2011 and October 2012.

Inclusion criteria were: (1) patients aged ≥ 18 yrs; (2) single missing tooth with adjacent teeth present; (3) diagnosis of health or history of generalized chronic or generalized aggressive periodontitis (Armitage, 1999) previously treated with at least 1 year of supportive periodontal therapy (SPT) – patients of generalized chronic or aggressive periodontitis had to be treated by the own group and had to participate in SPT in the Periodontology Clinic of Piracicaba Dental School; and (4) signing of the informed consent. Exclusion criteria were: (1) the presence of systemic diseases that possibly affect the healing process, e.g., diabetes; (2) smoking habits; (3) no adherence to SPT; (4) antibiotic therapy within 6 months prior to implant placement; (5) women who were pregnant or lactating; (6) the absence of keratinized tissue at the implant site – it could be interfere in hygiene around implants; (7) the need for antibiotic prophylaxis or post-operative antibiotic coverage; (8) the need for a simultaneous hard- or soft-tissue graft; (9) untreated periodontitis; and (10) inability or unwillingness to comply with study procedures and follow-up visits. Exit criteria were: (1) voluntary withdrawal; (2) non-compliance with study procedures or visits; (3) development of systemic or oral diseases requiring antibiotic

therapy; and (4) development of peri-implant infection/alteration requiring surgical intervention.

All those enrolled in this study received explanations about the possibilities of oral rehabilitation and provided signed written informed consent prior to entering the study. The patients were divided into three groups according to their periodontal history, as follows:

HGAgP group (14) – those who had a history of generalized aggressive periodontitis;

HGCP group (20) – those who had a history of generalized chronic periodontitis; and

HH group (15) – those who had no history of periodontitis and presented tooth loss due to caries, trauma or endodontic reasons.

Before patients entered the study, molds were made, and casts were provided for diagnostic waxing of each clinical case. Tomographic examinations were analyzed, and a custom-made multifunctional acetate stent was manufactured for surgical guidance and clinical evaluations. Patients were examined monthly after implant surgery up to six months after implants were loaded. During these visits, supportive periodontal treatment was performed. According to the patient's needs, oral hygiene instruction, supragingival calculus removal, prophylaxis with rubber cups, and subgingival debridement of pockets presenting PD ≥ 5 mm and BOP were performed.

Calibration

Three random patients presenting with GAgP were selected so that the examiner could perform the calibration. The designated examiner (HFV) measured relative clinical attachment level (rCAL) and probing depth (PD) of teeth in all three patients twice within 24 h, with an interval of ≥ 1 h between examinations. The intraclass correlation was calculated for each parameter, resulting in 90% reproducibility for relative CAL and 91% for PD. These patients did not enter the clinical trial.

Surgical Protocol

Before surgery, each patient's oral cavity was rinsed with 0.12% chlorhexidine for 1 min. Intraoral anti-inflammatory therapy consisting of 4 mg dexamethasone was provided 1 h before surgery.

All implants included in this study were manufactured by Straumann (Institut Straumann AG, Basel, Switzerland). The implants were all of the tissue-level type (standard plus) with sandblasted acid-etched (SLA) surfaces. Screw-type dental implants were placed by the same operator (TTV, instructed by MZC). After local anesthesia (4% Articaine with epinephrine 1:100,000; DFL Indústria e Comércio S.A., Rio de Janeiro, RJ, Brazil), a midcrestal incision was made, and full-thickness buccal and palatal/lingual mucoperiosteal flaps were reflected. The implants were inserted according to a standard one-stage surgical protocol following the manufacturer´s recommendations and using the manufacturer-specified surgical burs. All implants were placed leaving 1.8 mm of polished collar in the supra-osseous condition (according to the manufacturer´s instructions).

After implant placement, a manufacturer-provided healing abutment was inserted, and soft tissues were sutured with interrupted non-resorbable sutures. Patients were instructed to rinse the surgical site with 0.12% chlorhexidine twice a day for 7 days. Sutures were removed 7 days after surgery, and patients were instructed to resume their usual mechanical oral hygiene.

Prosthetic Rehabilitation

All clinical prosthetic procedures were performed by the same operator (MCCG, instructed by FHBA). The prosthetic procedures began 2 months after surgery. Metalloceramic screw-retained prostheses were manufactured by diagnostic waxing with Syn Octa components (Straumann). Implants were loaded with single crowns 3 months after surgery, and adjustments were made following the patients' maximum habitual intercuspation.

Clinical Parameters

Full-mouth clinical evaluations were obtained by means of a manual probe (PCPUNC 15®, HuFriedy, Chicago, IL, USA) before implant placement, at the day of prosthesis installation, and 1, 3, and 6 months after implant loading.

The following clinical parameters were evaluated at implant and tooth sites: (1) the presence of plaque, according to Ainamo and Bay (1975); (2) bleeding on probing (BOP), according to Mühlemann and Son (1971); (3) probing depth (PD); (4) relative gingival margin position (rGMP), the distance from the gingival margin to the stent margin; and (5) relative clinical attachment level (rCAL), the distance from the bottom of the pocket to the stent margin. Relative CAL and rGMP were obtained by use of the measurement stent.

At the time of implant placement, insertion torque (manual torquimeter; Straumann) and implant stability were obtained. Implant stability was also observed immediately before implant loading. Implant stability was measured via resonance frequency analysis (RFA) with the Osstell ISQ device (Osstell, Gothenburg, Sweden). A transducer (Smartpeg; Osstell) was manually screwed to the implant. The probe was held close to it in a mesio-distal direction during the evaluation. For improved precision and assessment of repeatability, two additional implant stability measurements were obtained. A single representative implant stability value was computed by the averaging of the three values.

Radiographic Evaluation

Radiographic stents were manufactured to standardize the x-ray incidence. The long-cone parallel technique was performed 7 days after implant surgery, at implant loading, and 6 months thereafter. The periapical films were automatically processed (Gendex GXP Dental X-ray Processor; Gendex Dental Systems, Hatfield, PA, USA) and digitized by a scanner (HP model G4050; Hewlett-Packard Development Company, L.P.

Palo Alto, CA, USA). All radiographs were evaluated by the same calibrated operator (MAGP; intraclass correlation of 0.91) blinded for clinical conditions.

Linear dimensions were calibrated to account for any distortion and magnification resulting from the imaging process. This was conducted by the use of a wire with known length associated with the radiographic stent. Measurements were recorded from the implant shoulder to the most coronal visible bone-to-implant contact on the mesial and distal sides of each implant, with UTHSCSA ImageTool software (Version 3.0). We computed a single representative bone-to-implant contact value by averaging both measurements.

Microbiologic and Immunologic Parameters

Peri-implant biofilm samples were collected from implant sites. Following supragingival biofilm removal and relative isolation with cotton rolls, four sterile paperpoints (#40) were inserted into the bottoms of the peri-implant sulci for 30 s (mesial, distal, lingual, and buccal). The paperpoints were placed in sterile tubes containing 300 μL of 0.5-mM Tris-EDTA and stored at -20^{o}C until further processing. Biofilm samples were collected at implant loading, and 1, 3, and 6 months thereafter.

Peri-implant crevicular fluid (PICF) was collected from mesial and distal sides of each implant after relative isolation and gentle drying. PICF was collected by the insertion of filter paper strips (Periopaper, Oraflow, Plainview, NY, USA) into the peri-implant sulci until the examiner perceived a slight resistance. The strips were maintained in place for 15s. The fluid volume was measured with a calibrated electronic device (Periotron 8000; Oraflow), and the strips were placed in sterile tubes containing 400 μL phosphate-buffered saline (PBS) with 0.05% polysorbate 20 (Tween 20, Sigma-Aldrich, St. Louis, MO, USA). Both peri-implant biofilm and PICF samples were immediately stored at -20^{o}C. Peri-implant crevicular fluid was collected 15 days after implant surgery, at implant loading, and 6 months thereafter.

Microbiologic Evaluation

All microbiologic and immunologic parameters were evaluated by the same operator (TT) blinded for clinical conditions. The presence and concentration of *Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf),* and *Aggregatibacter actinomycetemcomitans (Aa)* were evaluated with specific primers reported in the literature (Del Peloso Ribeiro et al., 2008; Casarin et al., 2010) by a real-time polymerase chain-reaction (PCR) technique.

Initially, DNA was extracted from the peri-implant biofilm with a DNA extraction kit (QIAamp DNA Mini Kit; Qiagen Inc., Valencia, CA, USA). Real-time PCR was performed with the hot-start reaction mix for PCR (FastStart DNA Master SYBR Green I; Roche Diagnostics, Mannheim, Germany). The concentration of DNA used in each run was 10 mg/mL. The amplification profiles were as follows: 95/10, 55/5, 72/4 [temperature (1°C)/time (s)], and 40 cycles for *Pg*; 95/10, 46/5, 72/5, and 45 cycles for *Tf*; and 95/10, 55/5, 72/3, and 40 cycles for *Aa*. Melting peaks were used to determine the specificity of the PCR.

Absolute quantification of target bacteria in clinical samples was performed with *Pg* (ATCC 33277), *Tf* (ATCC 43037), and *Aa* (JP2) as controls. Standard curves were made with these controls and used to convert cycle threshold scores into the number of bacterial cells, using controls with known amounts of bacterial-specific DNA. The level of detection was set at 10^3 bacteria/plaque sample for all target bacteria. The determination of DNA content in controls was based on the genome size of each bacteria and the mean weight of one nucleotide pair.

GCF Cytokine Levels and Bone Markers

Cytokine levels [granulocyte macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF-α)] in GCF were determined with the high-sensitivity human cytokine 10-plex

(Millipore Corporation, Billerica, MA, USA). Assays were carried out according to the manufacturer's recommendations with the MAGPIX® instrument (MiraiBio, Alameda, CA, USA). The samples were individually analyzed (each pocket separately), and concentrations were estimated from the standard curve according to a five-parameter polynomial equation with Xponent software (Millipore Corporation). The mean concentration of each marker was calculated based on the individual as a statistical unit and expressed as pg/mL.

The levels of the bone markers (OPG and RANKL) were determined with the LUMINEX/MAGPIX® system (HBN1A-51K and HCCBP1MAG-58K; Millipore Corporation). The samples were analyzed individually, and the levels were estimated based on a 5-parameter polynomial curve (Xponent software, Millipore Corporation). All results were adjusted for peri-implant crevicular fluid volume collected in each implant, and values were expressed in pg/mL.

Statistical Analysis

The analyses were performed by a blinded examiner (RCVC) who did not know the patients' status before the results. Only data from patients complying with all the evaluations were used in the statistical analysis. The analyses were performed with SAS 9.1 (SAS Software; SAS Institute, Cary, NC, USA) and Bioestat 5.0 (Instituto Mamirauá, Belém, PA, Brazil) with a significance level of 0.05.

Numeric variables such as patient age, implant stability, PD, rCAL, rGMP, plaque index (PI), BOP, radiographic bone loss, and microbiological and immunological variables were initially evaluated by the Shapiro-Wilk test (for normality). Those presenting a Shapiro-Wilk p-value > 0.05 were analyzed by ANOVA followed by Tukey's HSD test. Those presenting a Shapiro-Wilk p-value ≤ 0.05 were analyzed by the Friedman test (intragroup comparisons) and Kruskal-Wallis/Dunn tests (intergroup comparisons).

Comparisons among groups in terms of gender, frequency of implant length, implant diameter, torque insertion, presence of implant plaque, and implant BOP were performed by Fisher's Exact test.

5 RESULTS/*RESULTADOS*

Clinical Results

The patients' follow-up period is shown in Fig. 1. Four patients were excluded from the study during the 3-month interval between implant placement and prosthesis installation. One patient withdrew from the study, and three patients used systemic antibiotics because of health problems. Thus, 45 patients completed the six-month follow-up period, 13 of those presenting a history of generalized aggressive periodontitis, 18 presenting generalized chronic periodontitis, and 14 presenting no history of periodontitis.

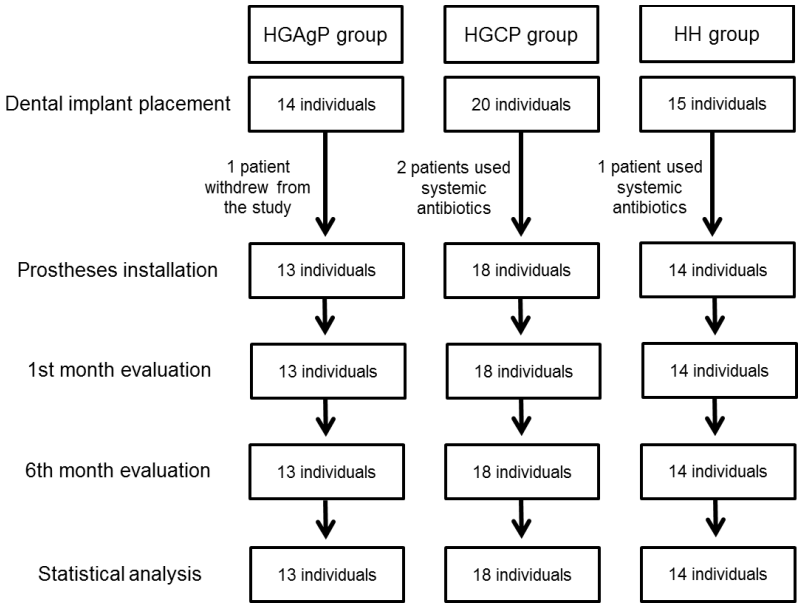

Figure 1 – Flow chart of the study. HGAgP = history of generalized aggressive periodontitis. HGCP = history of generalized chronic periodontitis. HH = no history of periodontitis.

In analyses of the demographics and clinical parameters (Table 1), no differences among groups were found in terms of gender, initial full-mouth PI, and BOP ($p > 0.05$). Patients' ages differed among groups, so those in the HGAgP group presented as 33.31 ± 3.82 yrs old at baseline, which was lower than the age presented by those in the HGCP and HH groups (49.89 ± 6.84 and 45.36 ± 11.70 yrs old, respectively) ($p < 0.05$). Those in the HH group presented lower values ($p < 0.05$) of clinical parameters such as full-mouth PD, rCAL, and rGMP compared with those in the HGAgP and HGCP groups.

Table 1 – *Study population demographic and clinical parameters evaluated before dental implant insertion*

Parameter	HGAgP Group	HGCP Group	HH Group
Age (yrs – mean ± SD)	33.31 ± 3.82 b	49.89 ± 6.84 a	45.36 ± 11.70 a
Gender - Woman – n (%)	11 (84.62%)	13 (72.22%)	8 (57.14 %)
p-value	0.2988		
Full-mouth PD (mm - mean ± SD)	2.42 ± 0.29 a	2.32 ± 0.30 a	2.06 ± 0.29 b
Full-mouth rCAL (mm - mean ± SD)	5.54 ± 0.99 a	5.48 ± 0.81 a	4.57 ± 0.69 b
Full-mouth rGMP (mm - mean ± SD)	3.12 ± 0.82 ab	3.17 ± 0.65 a	2.51 ± 0.64 b
Full-mouth PI (mm - mean ± SD)	18.86 ± 14.76 a	20.18 ± 13.67 a	26.07 ± 12.66 a
Full-mouth BOP (mm - mean ± SD)	17.15 ± 7.76 a	17.29 ± 7.41 a	17.68 ± 7.00 a

Distinct lowercase letters in a row indicate statistically significant difference by one-way ANOVA/Tukey's HSD test ($p < 0.05$).Gender parameter frequencies were analyzed by Fisher's Exact test.

Probing depth (PD), relative clinical attachment level (rCAL), relative gingival margin position (rGMP), plaque index (PI), and bleeding on probing (BOP).

According to the characteristics of the implants used during the study, all groups received implants 8, 10, and 12 mm long. In all groups, the 12-mm-long implant presented low frequency of use (23.08% HGAgP, 16.67% HGCP, and 13.33% HH). Implant lengths of 8 mm and 10 mm were the most common in this study, regardless of the

patient's periodontal history. No differences among groups were observed for frequency of implant length (Table 2).

Table 2 presents a comparison among groups for the frequencies of indication of each implant diameter. There was no difference among groups for the frequencies of 3.3-, 4.1-, and 4.8-mm-diameter implants used in each group ($p > 0.05$).

The insertion torque was divided into 3 classes: less than or equal to 15N, between 15N and 35N, and more than or equal to 35N. When frequencies of torque found in each group were considered, no differences were obtained among the groups (Table 2).

Table 2 – Comparison among groups in terms of the frequencies of implant length (mm), implant diameter (mm), and torque obtained at the time of implant placement (N)

Parameter		HGAgP Group n=13	HGCP Group n=18	HH Group n=14	p-value
Implant length	**8 mm – n (%)**	4 (30.77%)	8 (44.44%)	7 (46.67%)	0.9190
	10 mm – n (%)	6 (46.15%)	7 (38.89%)	6 (40.00%)	
	12 mm – n (%)	3 (23.08%)	3 (16.67%)	2 (13.33%)	
Implant diameter	**3.3 mm – n (%)**	3 (23.08%)	5 (27.78%)	1 (6.67%)	0.3455
	4.1 mm – n (%)	9 (69.23%)	9 (50.00%)	9 (60.00%)	
	4.8 mm – n (%)	1 (7.69%)	4 (22.22%)	5 (33.33%)	
Insertion torque	**≥ 35 N**	7 (53.85%)	9 (50.00%)	4 (26.67%)	0.5337
	15 < torque <35	4 (30.77%)	4 (22.22%)	6 (40.00%)	
	≤ 15N	2 (15.38%)	5 (27.78%)	5 (33.33%)	

Frequencies were compared by Fisher's Exact test.

Comparisons of initial implant stability measured by the Osstell ISQ device are shown in Table 3. The intergroup comparisons at the implant insertion and at the prosthesis installation did not show differences among the patient groups ($p > 0.05$). Nonetheless, the

intragroup analyses showed higher values of implant stability at the loading time than at surgery, in all groups ($p < 0.05$).

Table 3 – Comparisons among groups in terms of implant stability (ISQ), measured by the Osstell ISQ device, obtained at implant insertion and at implant loading.

Time	HGAgP Group	HGCP Group	HH Group
Implant insertion	72.70 (± 4.23) Ba	71.96 (± 10.93) Ba	74.59 (± 6.20) Ba
Implant loading	78.92 (± 4.82) Aa	79.30 (± 3.67) Aa	82.23 (± 5.87) Aa

Distinct uppercase letters in a column and distinct lowercase letters in a row indicate statistically significant differences by ANOVA/Tukey's HSD test ($p \leq 0.05$).

During the study period, those in the HH group presented PD and rCAL values lower than those in the HGAgP and HGCP groups (Table 4, Figure 2). No intragroup differences ($p > 0.05$) were seen during the follow-up period. Thus, stable periodontal conditions were maintained in all groups from the time of dental implant surgery until the 6^{th} month after loading.

In terms of the implant sites, no difference among the HGAgP, HGCP, and HH groups was observed during the study period (Table 5). Probing-depth intragroup differences ($p < 0.05$) were seen in the HGCP and HH groups when baseline levels were compared with those at 6 months after loading. When the gain or loss of PD during the study period was compared, no difference was observed among the groups ($p > 0.05$).

Table 4 – Inter- and intragroup comparisons in terms of averages (± SD) of full-mouth probing depth (PD), full-mouth relative clinical attachment level (rCAL), and relative gingival margin position (rGMP) obtained during the study.

Parameters	Baseline	Prosthesis Installation	1 Month of Loading	3 Months of Loading	6 Months of Loading
Full-mouth PD (mm)					
HGAgP	2.42 ± 0.29 Aa	2.36 ± 0.23 Aa	2.33 ± 0.28 Aa	2.38 ± 0.35 Aa	2.28 ± 0.31 Aa
HGCP	2.32 ± 0.30 Aa	2.23 ± 0.28 ABa	2.18 ± 0.27 Ba	2.22 ± 0.31 Ba	2.24 ± 0.33 ABa
HH	2.06 ± 0.29 Ba	2.11 ± 0.22 Ba	2.09 ± 0.19 Ba	2.10 ± 0.20 Ba	2.17 ± 0.20 Ba
Full-mouth rCAL (mm)					
HGAgP	5.54 ± 0.99 Aa	5.78 ± 1.11 Aa	5.85 ± 1.18 Aa	6.06 ± 1.58 Aa	5.73 ± 1.10 Aa
HGCP	5.48 ± 0.81 Aa	5.62 ± 0.78 ABa	5.57 ± 0.84 ABa	5.60 ± 0.91 ABa	5.63 ± 0.84 Aa
HH	4.57 ± 0.69 Ba	4.89 ± 0.70 Ba	4.80 ± 0.71 Ba	4.85 ± 0.72 Ba	4.94 ± 0.73 Aa
Full-mouth rGMP (mm)					
HGAgP	3.12 ± 0.82 ABa	3.42 ± 1.03 Aa	3.53 ± 1.11 Aa	3.68 ± 1.34 Aa	3.45 ± 0.96 Aa
HGCP	3.17 ± 0.65 Aa	3.46 ± 0.76 Aa	3.38 ± 0.75 Aa	3.37 ± 0.77 Aa	3.40 ± 0.74 Aa
HH	2.51 ± 0.64 Ba	2.78 ± 0.69 Aa	2.71 ± 0.68 Aa	2.75 ± 0.71 Aa	2.76 ± 0.73 Aa

In PD, distinct uppercase letters in a column and distinct lowercase letters in a row indicate statistically significant differences by ANOVA/Tukey's HSD test ($p \leq 0.05$). In rCAL and rGMP, distinct uppercase letters in a column indicate statistically significant differences by the Kruskal Wallis/Dunn tests ($p < 0.05$), and distinct lowercase letters in a row indicate statistically significant differences by the Friedman test ($p \leq 0.05$).

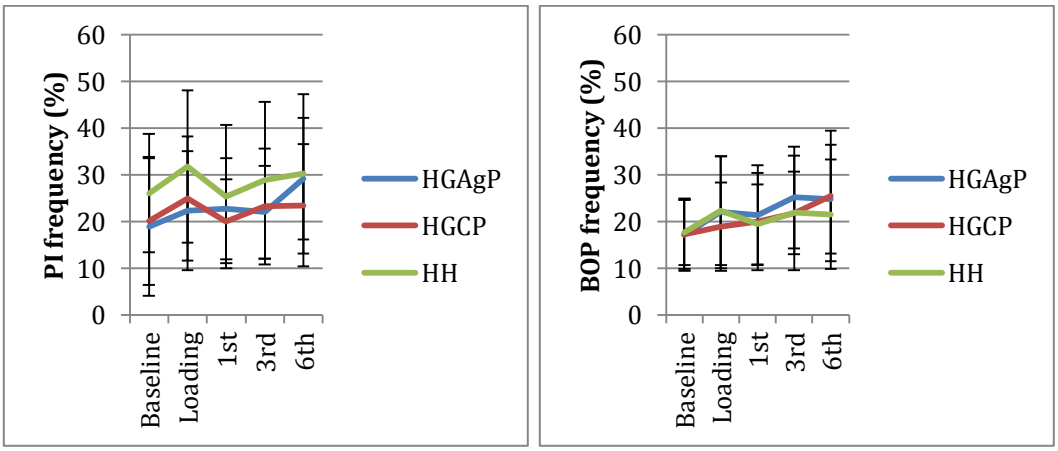

Figure 2 – Frequency (%) of full-mouth plaque index (PI) and bleeding on probing (BOP) during follow-up period. Both PI and BOP showed no differences in intra- or intergroup comparisons by ANOVA/ Tukey's HSD test (p > 0.05).

For rCAL at implant sites, only the HGAgP group showed differences among the evaluations (Table 5). In this group, the values obtained at the 3rd and 6th months of evaluation (7.97 ± 2.07 and 7.89 ± 2.26 mm, respectively) were higher than those obtained at the 1st month of loading (7.46 ± 2.00 mm) ($p < 0.05$). This difference was also found when gain or loss in the rCAL parameter (Table 5) was compared in the HGAgP group. In the first month, there was a gain of 0.24 ± 0.58 mm compared with baseline, and losses of 0.26 ± 0.71 and 0.24 ± 0.58 mm were seen at the 3rd and 6th months. The HGCP and HH groups did not show intragroup differences ($p > 0.05$).

rGMP analyses showed no difference among groups in the averages obtained after implant loading. However, the analysis of gain/loss of rGMP presented intra- and intergroup differences. Only the HGAgP group presented intragroup differences between gain at the 1st month (0.15 ± 0.50 mm) and loss at the 3rd month after loading (0.19 ± 0.55 mm) ($p < 0.05$). Inter-group comparisons showed differences among all groups at the 3- and 6- month evaluations. At the 3rd month after loading, the HGAgP group presented loss of 0.19 ± 0.55 mm, and the HGCP and HH groups presented gains of 0.25 ± 0.41 and 0.18

Table 5 – Intra- and intergroup comparisons of average (± SD) of clinical parameters [probing depth (PD), relative clinical attachment level (rCAL), and relative gingival margin position (rGMP)] obtained at implant sites and the clinical gain/loss in each parameter during the study.

Parameter	Time				Δ 1st – Prosthesis Inst.	Δ 3rd – Prosthesis Inst.	Δ 6th – Prosthesis Inst.
	Prosthesis Installation	1st month	3rd month	6th month			
Implant PD (mm)							
HGAgP	2.59 ± 0.47 Aa	2.51 ± 0.44 Aa	2.64 ± 0.50 Aa	2.65 ± 0.43 Aa	-0.08 ± 0.36 Aa	0.04 ± 0.46 Aa	0.05 ± 0.46 Aa
HGCP	2.51 ± 0.43 Ab	2.61 ± 0.41 Aab	2.69 ± 0.47 Aab	2.83 ± 0.41 Aa	0.10 ± 0.44 Aa	0.18 ± 0.39 Aa	0.32 ± 0.49 Aa
HH	2.85 ± 0.59 Ab	3.04 ± 0.56 Aab	3.13 ± 0.58 Aab	3.27 ± 0.75 Aa	0.23 ± 0.45 Aa	0.31 ± 0.43 Aa	0.47 ± 0.52 Aa
Implant rCAL (mm)							
HGAgP	7.71 ± 2.00 Aab	7.46 ± 2.00 Ab	7.97 ± 2.07 Aa	7.89 ± 2.26 Aa	-0.24 ± 0.58 Ab	0.26 ± 0.71 Aa	0.24 ± 0.58 Aa
HGCP	7.05 ± 1.61 Aa	7.08 ± 1.53 Aa	6.91 ± 1.70 Aa	7.17 ± 1.60 Aa	0.02 ± 0.59 Aa	-0.07 ± 0.54 Aa	0.11 ± 0.53 Aa
HH	6.26 ± 2.59 Aa	6.29 ± 2.45 Aa	6.24 ± 2.34 Aa	6.46 ± 2.40 Aa	0.13 ± 0.65 Aa	0.08 ± 0.76 Aa	0.32 ± 0.81 Aa
Implant rGMP							
HGAgP	5.03 ± 1.79 Aa	4.87 ± 1.73 Aa	5.21 ± 1.68 Aa	5.08 ± 1.86 Aa	-0.15 ± 0.50 Ab	0.19 ± 0.55 Aa	0.12 ± 0.56 Aab
HGCP	4.54 ± 1.31 Aa	4.46 ± 1.44 Aa	4.22 ± 1.46 Aa	4.34 ± 1.57 Aa	-0.08 ± 0.37 Aa	-0.25 ± 0.41 Ba	-0.21 ± 0.47 Ba
HH	3.56 ± 1.98 Aa	3.42 ± 1.89 Aa	3.30 ± 1.83 Aa	3.37 ± 1.89 Aa	-0.06 ± 0.50 Aa	-0.18 ± 0.64 Ba	-0.11 ± 0.61 ABa

In comparisons of clinical implant parameters and clinical gain/loss, distinct uppercase letters in a column and distinct lowercase letters in a row indicate statistically significant differences by ANOVA/Tukey HSD

tests ($p \leq 0.05$). In the clinical gain/loss table, negative values indicate gains in clinical parameters, and positive values indicate loss in clinical parameters.

± 0.64 mm, respectively ($p < 0.05$). At the 6-month evaluation, HGAgP presented a loss of 0.12 ± 0.56 mm, and HGCP presented a gain of 0.21 ± 0.47 mm ($p < 0.05$).

The frequencies of the numbers of implants presenting visible plaque during the evaluations are shown in Fig. 3. The intergroup comparisons of these frequencies, performed at each time period, showed no statistically significant differences ($p > 0.05$). The frequencies of implants presenting at least one site with BOP are also shown in Fig. 3. These frequencies were high in all evaluations, and no statistically significant differences were seen in intra- or intergroup comparisons ($p > 0.05$).

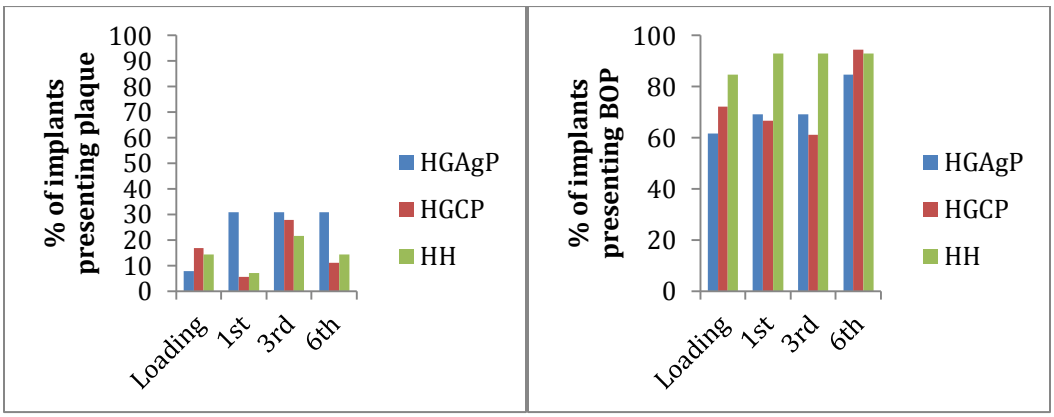

Figure 3 – Illustration of frequency of implants presenting visible plaque during the evaluations and frequency of implants presenting at least 1 site with bleeding on probing (BOP). No differences between the frequencies presented by each group were observed by Fisher´s Exact test ($p>0.05$).

Radiographic Results

The results of radiographic analysis showed no differences among groups ($p > 0.05$), but in all groups, intragroup comparisons already presented statistically significantly higher distances ($p < 0.05$) from platform to bone contact at the time of implant loading ($p < 0.05$). The quantity of bone resorption did not differ among groups at the time of implant loading or at the 6-month post-loading evaluation. Only the HH group presented higher values of resorption at the 6-month evaluation (1.14 ± 1.11 mm) compared with that at the time of implant loading (0.84 ± 0.91 mm).

Table 6 – Comparison of the radiographic distances (mm) measured from the implant platform and the first implant-bone contact and the gain/loss bone comparisons at 3 distinct time-points of the study.

Group	Time			Δ Implant Loading – Implant Insertion	Δ 6-month Loading – Implant Insertion
	Implant Insertion	Implant Loading	6 Months after Loading		
HGAgP	1.15 ± 1.03 Ab	1.70 ± 1.16 Aa	1.91 ± 1.20 Aa	0.54 ± 0.88 Aa	0.77 ± 1.32 Aa
HGCP	1.61 ± 1.11 Ab	2.27 ± 1.00 Aa	2.37 ± 0.98 Aa	0.67 ± 0.97 Aa	0.80 ± 0.94 Aa
HH	1.28 ± 0.74 Ab	2.12 ± 0.48 Aa	2.43 ± 0.67 Aa	0.84 ± 0.91 Ab	1.14 ± 1.11 Aa

In reference to distance, distinct uppercase letters in a column indicate statistically significant differences by Kruskal-Wallis/Dunn tests ($p < 0.05$), and distinct lowercase letters in a row indicate statistically significant differences by Friedman´s test ($p \leq 0.05$). In reference to resorption analysis, distinct uppercase letters in a column indicate statistically significant differences by Kruskal-Wallis/Dunn tests ($p < 0.05$), and distinct lowercase letters in a row indicate statistically significant differences by Wilcoxon´s test ($p < 0.05$).

Immunologic Results

The evaluation of inflammatory markers showed no differences among groups for all variables (Table 7) except for IL-4, which, at 6 months after loading, presented lower levels in those in the HGAgP and HGCP groups compared with those in the HH group. Only those in the HGAgP group presented differences in IL-6 levels during the study. For

this group, at the time of implant loading, the IL-6 level was higher ($p < 0.05$) than at 15 days after surgery and 6 months after implant loading.

Table 7 – Intra- and intergroup comparisons of immunological variables: GM-CSF, IFNγ, IL-10, IL-1β, IL-8, IL-6, TNFα, and IL-4.

Inflammatory Markers	Time		
	15 Days after Surgery	Loading	6 Months after Loading
GM-CSF (pg/mL)			
HGAgP	0.10 ± 0.15 Aa	0.27 ± 0.32 Aa	0.14 ± 0.12 Aa
HGCP	0.12 ± 0.21 Aa	0.17 ± 0.17 Aa	0.20 ± 0.23 Aa
HH	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa
IFNγ (pg/mL)			
HGAgP	0.46 ± 0.86 Aa	0.99 ± 1.83 Aa	0.09 ± 0.16 Aa
HGCP	0.55 ± 1.00 Aa	1.04 ± 1.71 Aa	0.70 ± 1.29 Aa
HH	0.71 ± 1.47 Aa	0.52 ± 0.69 Aa	0.94 ± 1.82 Aa
IL-10 (pg/mL)			
HGAgP	2.08 ± 1.22 Aa	3.31 ± 2.62 Aa	1.54 ± 1.69 Aa
HGCP	2.09 ± 2.48 Aa	3.83 ± 3.85 Aa	3.19 ± 3.37 Aa
HH	0.97 ± 1.84 Aa	2.06 ± 3.21 Aa	1.22 ± 1.90 Aa
IL-1β (pg/mL)			
HGAgP	4.01 ± 4.71 Aa	2.84 ± 3.85 Aa	0.99 ± 1.38 Aa
HGCP	2.28 ± 3.05 Aa	2.54 ± 4.20 Aa	2.39 ± 3.34 Aa
HH	0.78 ± 0.96 Aa	1.01 ± 1.91 Aa	3.32 ± 4.02 Aa

For each immunological variable, distinct uppercase letters in a column indicate statistically significant differences by Kruskal Wallis/Dunn tests ($p < 0.05$), and distinct lowercase letters in a row indicate statistically significant difference by Friedman´s test ($p \leq 0.05$).

Cont. table 7 – Intra- and intergroup comparisons of immunological variables: GM-CSF, IFNγ, IL-10, IL-1β, IL-8, IL-6, TNFα, and IL-4.

Inflammatory Markers	Time		
	15 Days after Surgery	Loading	6 Months after Loading
IL-8 (pg/mL)			
HGAgP	185.13 ± 123.18 Aa	181.68 ± 160.17 Aa	110.76 ± 100.03 Aa
HGCP	180.87 ± 219.28 Aa	262.01 ± 266.13 Aa	179.45 ± 243.83 Aa
HH	114.20 ± 159.08 Aa	125.17 ± 157.94 Aa	59.73 ± 94.38 Aa
IL-6 (pg/mL)			
HGAgP	0.32 ± 0.26 Ab	1.51 ± 3.75 Aa	0.40 ± 0.44 Ab
HGCP	0.95 ± 1.17 Aa	0.40 ± 1.01 Aa	0.70 ± 1.46 Aa
HH	0.28 ± 0.39 Aa	0.23 ± 0.35 Aa	0.21 ± 0.55 Aa
TNFα (pg/mL)			
HGAgP	0.08 ± 0.16 Ab	0.05 ± 0.11 Ab	0.40 ± 0.70 Aa
HGCP	0.04 ± 0.07 Ab	0.13 ± 0.31 Ab	1.10 ± 1.77 Aa
HH	0.03 ± 0.05 Ab	0.11 ± 0.28 Ab	1.00 ± 1.51 Aa
IL-4 (pg/mL)			
HGAgP	0.66 ± 0.45 Aa	0.89 ± 0.98 Aa	0.16 ± 0.25 Ba
HGCP	1.73 ± 2.26 Aa	0.90 ± 1.10 Aa	0.48 ± 1.12 Ba
HH	1.27 ± 1.91 Aa	1.26 ± 1.97 Aa	3.05 ± 5.07 Aa

For each immunological variable, distinct uppercase letters in a column indicate statistically significant differences by Kruskal Wallis/Dunn tests ($p < 0.05$), and distinct lowercase letters in a row indicate statistically significant difference by Friedman´s test ($p \leq 0.05$).

TNFα had similar responses in all groups (Table 7). The TNFα intragroup comparisons showed higher values ($p < 0.05$) at 6 months after implant loading, while lower values were seen at 15 days after surgery and at the time of implant loading.

The analyses of osteogenic markers such as OPG and RANKL presented differences among groups only at the time of implant loading in OPG levels. In this case, those in the HH group showed OPG levels higher ($p < 0.05$) than those in the HGAgP and HGCP groups. At the other measurement times, no intergroup differences were seen ($p > 0.05$).

Intragroup comparisons showed differences in the HGAgP group for the RANKL marker. The RANKL level at the time of loading was higher than that at 6 months after loading (Table 8). In terms of OPG levels, only those in the HH group presented lower levels ($p < 0.05$) at 15 days after surgery than at the time of implant loading and 6 months after loading.

Table 8 – Intra- and intergroup comparisons of osteogenic markers: OPG and RANKL.

Osteogenic Markers	**Time**		
	15 Days after Surgery	**Loading**	**6 Months after Loading**
OPG (pg/mL)			
HGAgP	8.6 ± 7.4 Aa	19.7 ± 8.9 Ba	22.2 ± 23.9 Aa
HGCP	3.9 ± 2.9 Aa	20.1 ± 10.8 Ba	12.8 ± 14.8 Aa
HH	5.0 ± 0.8 Ab	40.5 ± 10.0 Aa	42.2 ± 19.9 Aa
RANKL (pg/mL)			
HGAgP	7.4 ± 5.2 Aab	16.3 ± 7.9 Aa	6.1 ± 7.2 Ab
HGCP	8.7 ± 10.1 Aa	13.5 ± 16.1 Aa	2.7 ± 3.2 Aa
HH	2.2 ± 0.5 Aa	3.2 ± 1.8 Aa	3.1 ± 3.1 Aa

For each osteogenic variable, distinct uppercase letters in a column indicate statistically significant differences by Kruskal Wallis/Dunn tests ($p < 0.05$), and distinct lowercase letters in a row indicate statistically significant difference by Friedman´s test ($p < 0.05$).

Microbiologic Results

Table 9 –Amounts of A. actinomycetemcomitans, P. gingivalis, *and* T. forsythia *in peri-implant subgingival biofilm in patients either healthy or presenting a history of generalized aggressive periodontitis or generalized chronic periodontitis at the time of loading and after 1, 3, and 6 months of loading.*

Bacteria	Time			
	Implant Loading	**1st Month**	**3rd Month**	**6th Month**
Aa				
HGAgP	2.67 ± 3.01 Aa	2.71 ± 2.67 Aa	1.84 ± 2.52 Aa	2.32 ± 2.21 Aa
HGCP	1.62 ± 1.42 Aa	0.40 ± 0.69 Ba	0.40 ± 0.70 Aa	1.13 ± 1.11 Aa
HH	1.32 ± 1.87 Aa	0.32 ± 0.87 Ba	1.32 ± 1.87 Aa	1.33 ± 1.88 Aa
Pg				
HGAgP	1.22 ± 2.02 Ab	2.14 ± 1.93 Aab	3.19 ± 2.45 Aab	3.66 ± 2.50 Aa
HGCP	0.84 ± 1.30 Ab	3.25 ± 2.20 Aa	3.31 ± 1.72 Aa	3.41 ± 1.86 Aa
HH	1.33 ± 1.88 Aa	1.10 ± 1.56 Aa	2.21 ± 1.00 Aa	0.89 ± 0.78 Ba
Tf				
HGAgP	3.70 ± 2.90 Ab	4.64 ± 2.73 Aab	5.94 ± 0.87 Aa	3.49 ± 3.26 Ab
HGCP	2.77 ± 1.75 Aa	4.05 ± 3.18 Aa	4.79 ± 2.42 Aa	5.00 ± 2.54 Aa
HH	3.23 ± 2.04 Aa	4.84 ± 1.20 Aa	2.77 ± 3.91 Aa	3.57 ± 1.82 Aa

Distinct uppercase letters in a column indicate statistically significant difference by Kruskal Wallis/Dunn tests ($p < 0.05$), and distinct lowercase letters in a row indicate statistically significant difference by Friedman´s test ($p < 0.05$).

The microbiological evaluations for *Aa*, *Pg*, and *Tf* revealed differences among groups for *Aa* levels at the 1st month after implant loading (Table 9). At this time, those in

the HGAgP group showed *Aa* concentrations (2.71 ± 2.67) higher ($p < 0.05$) than those in the HGCP and HH groups. The *Pg* analyses showed differences among groups only at 6 months after implant loading, while those in the HGAgP and HGCP groups presented higher levels of *Pg* than those in the HH group (Table 9). No differences among groups were seen for *Tf* levels ($p > 0.05$).

Intragroup comparisons showed no differences among time evaluations for *Aa*. For *Pg* levels, those in the HGCP and HGAgP groups presented lower concentrations at implant loading ($p < 0.05$) and higher concentrations at 6 months after loading. In *Tf* analyses, only those in the HGAgP group showed differences among the evaluations, when the levels were higher at 3 months after loading and lower at implant loading and 6 months after loading.

6 DISCUSSION/*DISCUSSÃO*

Healthy individuals and those with aggressive and chronic periodontitis have shown different clinical and radiographic outcomes after dental implant therapy, and long-term evaluations have shown increased bone resorption, more risk for the development of mucositis and peri-implantitis, and lower survival and success rates for patients presenting a history of aggressive periodontitis (Al-Zahrani, 2008; Kim & Sung, 2012). Since this could be related to microbiologic and immunologic parameters, the present prospective, parallel, controlled clinical trial investigated the clinical, microbiological, and immunological parameters of dental implant rehabilitation in healthy partially edentulous patients and in those with a history of generalized aggressive and generalized chronic periodontitis.

Initially, the patients were divided into three groups according to their history of previous periodontal disease. Age differences were observed among groups, but the ages of those with aggressive (33.31 ± 3.82 yrs old) and chronic (49.89 ± 6.84 yrs old) periodontitis were in agreement with classification system for periodontal diseases and conditions (Armitage, 1999).

Clinical parameters showed lower values for full-mouth PD, rCAL, and rGMP in the HH group compared with those in the HGAgP and HGCP groups throughout the study. These differences were expected, since those in the HGAgP and HGCP groups had previously experienced tissue losses due to generalized periodontitis. In spite of these differences among groups, all patients were enrolled in supportive periodontal treatment which could be confirmed by full-mouth parameters of health during the study and the stability of full-mouth periodontal conditions maintained in each group.

An expected difference among groups would be in implant length, diameter, and torque insertion. Periodontal diseases can promote severe attachment loss and bone resorption, reducing the quantity of bone available for implant insertion. The literature,

however, shows that periodontitis is not the only cause of tooth loss. Montandon et al. (2012) reported caries and periodontitis as statistically significant reasons for tooth loss compared with factors such as endodontic problems, eruption problems, prosthetics, trauma, orthodontics, and occlusal problems. In periodontally healthy patients, the time of tooth loss could influence vertical and horizontal alveolar ridge resorption (Tan et al., 2012) and could explain why, in the present study, no among-group differences were observed relative to the implants used. The same 8-, 10-, and 12-mm implant lengths and the same 3.3-, 4.1-, and 4.8-mm implant diameters were used in each group. Also, the same frequencies of torque insertion measurements were observed.

Another characteristic evaluated was resonance frequency analysis (RFA), and among-group comparisons were made. This study showed, at implant insertion, close values of RFA among groups, without statistically significant differences. The absence of differences among groups observed at implant loading reinforced the contention that, at implant placement and at implant loading, periodontally healthy patients and those with aggressive and chronic periodontitis share the same RFA. Studies have shown increased RFA readings after implant healing (Al-Juboori et al., 2013; Shokri et al., 2013), probably due to osseointegration. In agreement with this, in this study, RFA values increased from 72.70, 71.96, and 74.59 ISQ units (HGAgP, HGCP, and HH groups, respectively) at implant insertion to 78.92, 79.30, and 82.23 ISQ units (HGAgP, HGCP, and HH groups, respectively) at implant loading.

Although several previous studies have described a higher incidence of peri-implantitis and lower success rates in patients treated for periodontitis (Safii et al., 2010; Donos, 2012; Marrone et al., 2013), this study did not show additional deterioration around implants in the periodontitis groups. In terms of clinical parameters obtained at implant sites, no differences among groups were found. The parameters rCAL and rGMP did not show different values in inter- and intragroup comparisons.

During the 6 months after implant loading, the values of probing depth increased only around implants placed in the HGCP and HH groups, but at this time PD

values (HGAgP, 2.65 ± 0.43 mm; HGCP, 2.83 ± 0.41 mm; HH, 3.27 ± 0.75 mm) were similar to those reported by Cortelli et al. (2013) (3.02 ± 1.07 mm for healthy and 3.42 ± 1.18 mm for mucositis implant sites), by Dierens et al. (2013) (3.8 ± 1.4 mm after 16 to 22 years of function), and by Yaghobee et al. (2013) (3.31 ± 1.01 mm). It is probable that the PD values increased due to the higher frequency of BOP found during the follow-up period.

In this study, in spite of monthly supportive periodontal treatment, all groups presented at least a 60% frequency of implant bleeding, and this constant inflammation could influence the probing depth during evaluations. Other studies also reported higher frequencies of implants presenting at least one site with BOP (DeAngelo et al., 2007; Lachmann et al., 2012).

In spite of high implant BOP frequencies, little bone resorption around implants was observed up to 6 months after loading. Among the many hypotheses that have been postulated as reasons for these early crestal bony changes, the establishment of an implant "biologic width" is one hypothesis that implicates peri-implant soft-tissue changes (Abrahamsson et al., 1996; Berglundh et al., 1996). Thus, the one-stage implant protocol used in this study could collaborate with the bone changes seen at implant loading. Bone resorption was similar in all groups, independent of periodontal history. After implant insertion, alveolar crest alterations were 0.77 ± 1.32 mm, 0.80 ± 0.94, and 1.14 ± 1.11 in the HGAgP, HGCP, and HH groups after 6 months of implant loading, and no difference was observed among groups. These changes in alveolar bone were in agreement with reports from other studies (Mengel & Flores-de-Jacoby, 2005; Mengel et al., 2007; Mengel et al., 2007; Al-Juboori et al., 2013; Yaghobee et al., 2013). Al-Juboori et al. (2013) found 0.98 ± 0.56 mm crestal bone loss in Straumann Standard Implants 12 weeks after implant insertion. Mengel and Flores-de-Jacoby (2005) found 1.14 mm of bone loss in patients with aggressive periodontitis, 0.86 mm in patients with chronic periodontitis, and 0.70 mm in periodontally healthy patients after 3 years of loading. Mengel et al. (2007) reported 1.29 mm of bone marginal bone loss in GAP patients and 0.71 mm in healthy patients after 3 years. In a ten-year evaluation, Mengel et al. (2007) reported 2.07 mm of marginal bone loss in the first year of function in GAP patients, and 1.13 mm in healthy patients.

Yaghobee et al. (2013) reported bone loss of 1.66 ± 1.06 mm in healthy patients, after 3 years of loading with Straumann implants.

The pathogens *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*, and *Tannerella forsythia* were detected in healthy and diseased implants (Casado et al., 2011; Cortelli et al., 2013), and it was demonstrated that, even after tooth loss, key periodontal pathogens remain, colonizing the oral cavity (Fernandes et al., 2010). Thus, it has been suggested that the bacteria which cause periodontal breakdown could migrate and colonize peri-implant sites (Quirynen et al., 2005). The mere presence of *Pi*, *Pg*, and *Aa* was correlated with higher probing depths, increased bleeding, and a higher gingival sulcus fluid rate (George et al., 1994), but Casado et al. (2011) indicated that the presence of these periodontal pathogens in peri-implant sulci does not necessarily lead to destruction or even inflammatory symptoms, but, rather, that a combination of factors, including genetics, inflammatory response, and occlusal overload, may be involved.

The present study investigated the presence of three periodontal pathogens and showed that *Aa*, *Pg*, and *Tf* were present around dental implants inserted into healthy periodontal patients and in patients presenting a history of periodontal disease. Differences among groups were observed at the 1st month after implant loading, when the *Aa* concentration was higher in the HGAgP group, and at 6 months after implant loading, when the *Pg* concentration was lower in the HH group.

Studies reported in the literature have shown higher *Aa* levels in patients with aggressive than in those with chronic periodontitis (Schacher et al., 2007; Casarin et al., 2010), which explains the difference among groups found at 1 month after implant loading. Regarding the *Pg* levels found in this study, the literature has reported a higher prevalence of *Pg* in those with chronic periodontitis than in periodontally healthy individuals (Cortelli et al., 2013), as well as higher *Pg* levels in patients with aggressive periodontitis (Casarin et al., 2010), so the higher levels of this bacterium in periodontitis groups at 6 months after implant loading is justified. In spite of this, no clinical or radiographic differences were

observed among groups, but it is important to note that the frequency of implants presenting BOP was elevated. A long-term follow-up should be done to investigate the consequences of microbiological presence, and other biofilm bacteria should be investigated to confirm the absence of differences among groups.

In terms of immunologic parameters, studies have suggested that peri-implant gingiva may be characterized by a higher pro-inflammatory state under apparent homeostatic conditions (clinically healthy tissues) (Nowzari et al., 2008), and cytokines such as IL-1β, TNFα, and IL-8 can be predictors of peri-implant destruction (Petkovic et al., 2010). Thus, the analysis of inflammatory factors is important, considering that a history of periodontitis is associated with peri-implant diseases, and an imbalance between pro- and anti-inflammatory cytokines in aggressive periodontitis has been suggested (Teles et al., 2010).

The present study shows intergroup differences only for interleukin-4 (IL-4) levels. Those in the HH group presented higher values of IL-4 than did those in the HGCP and HGAgP groups at 6 months after loading. IL-4, like interleukin-10 (IL-10), is an anti-inflammatory Th2 cytokine that inhibits disease progression in inflammation sites and has a fundamental role in mediating bone resorption (Ebersole et al., 1994). The IL-4 levels of those in the HGAgP and HGCP groups were similar to those found by Fonseca et al. (2012) in peri-implantitis deep-pocket sites. IL-10 levels did not differ among groups and did not present intragroup alterations, but the levels observed in this study were similar to those found by Fonseca et al. (2012) in mucositis sites.

Interestingly, interleukin-1beta (IL-1β) levels did not differ among groups. IL-1β is a pro-inflammatory polypeptide implicated in a wide range of biological processes, including inflammation, tissue breakdown, and tissue homeostasis (Tatakis et al., 1993; Ataoglu et al., 2002). Teles et al. (2010) suggested an imbalance between pro- and anti-inflammatory cytokines in aggressive periodontitis. Based on this, high levels of IL-1β were expected in this group compared with the HGCP and HH groups, but this did not occur. In spite of that, the level of IL-1β found in this study was greater than that found by

Fonseca et al. (2012) in peri-implantitis sites in healthy patients. Other pro-inflammatory cytokines also presented alterations.

Tumor necrosis factor alpha (TNFα) is a cytokine with a tendency to increase during the follow-up period. Even though no differences among groups were found, the values observed at the 6-month evaluation after implant loading were greater than those found previously. TNFα is a pro-inflammatory cytokine with the same function as IL-1β (Ataoglu et al., 2002), so its increase represents a risk for tissue breakdown due to osteoclastogenesis.

Other cytokines presenting time-related alterations included interleukin-6 (IL-6). In the HGAgP group, a significant increase was observed at implant loading, but this level returned to initial values after surgery. Interleukin-8, a potent chemotactic agent for neutrophils (Okada et al., 1998), presented high levels of expression in all groups. Although no differences among groups could be obtained, these values were higher than those reported by Fonseca et al. (2012) in mucositis sites, and were close to those reported by Petkovic et al. (2010) in early peri-implantitis sites, demonstrating a high concentration of pro-inflammatory factors.

The present study also investigated the osteogenic markers OPG and RANKL. RANKL (osteoclast differentiation factor) binds directly to RANK on the surfaces of pre-osteoclasts and osteoclasts, stimulating both the differentiation of osteoclast progenitors and the activity of mature osteoclasts (Lacey et al., 1998). Conversely, OPG, also known as osteoclastogenesis inhibitory factor, is a soluble circulating decoy receptor of RANKL that antagonizes the RANK-RANKL interaction and, therefore, promotes bone formation by inhibiting osteoclastogenesis (Bartold et al., 2010).

In this study, the OPG levels in those in the HH group increased after implant surgery, and greater levels of this protein were obtained at implant loading in those in the HH group, but this difference was not found 6 months after loading. OPG levels at implant loading and 6 months later, in all groups (HGAgP, 19.70 ± 8.9 and 22.2 ± 23.9 pg/mL; HGCP, 20.1 ± 10.8 and 12.8 ± 14.8 pg/mL; HH, 40.5 ± 10.0 and 42.2 ± 19.9 pg/mL), were

similar to those found by Rakic et al. (2012) in healthy (15.92 ± 8.98 pg/mL) and diseased implant sites (18.99 ± 9.96 pg/mL) with 2 years of function.

RANKL levels did not differ among groups, but in the HGAgP group, a statistically significant reduction in RANKL levels was observed after implant loading. In this group, a coincidence can be noted between the increased RANKL level and the point of greater bone alteration (0.54 ± 0.88 mm), but no additional bone loss was observed.

The results presented in this study must be carefully evaluated, since they explain only 6 months of follow-up evaluation. Until now, the results corroborated those of Kim & Sung (2012), that implant treatment in patients presenting a history of generalized aggressive periodontitis is not contraindicated, since adequate infection control and an individualized maintenance program can be ensured. Nevertheless, patients with generalized aggressive periodontitis are susceptible to inflammation, and dental implant rehabilitation is a challenge requiring longitudinal studies to confirm the results presented here.

7 CONCLUSION/*CONCLUSÃO*

Within the limitations of this study, it can be concluded that, 6 months after implant loading, *Pg* levels are greater in periodontitis patients, and IL-4 levels are greater in periodontally healthy individuals, but these conditions do not reflect clinical differences or additional bone resorption around implants placed in patients presenting a history of periodontal disease after a follow-up of 6 months.

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UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Implantes dentais osseointegrados em pacientes com histórico de periodontite agressiva e crônica. Avaliação clínica, microbiológica e imunoenzimática"**, protocolo nº 017/2010, dos pesquisadores Renato Corrêa Viana Casarin, Hugo Felipe do Vale, Márcio Zaffallon Casati e Tiago Taiete, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 30/10/2010.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Osseointegrated implants in patients with history of aggressive and chronic periodontitis. Clinical, microbiological and immunoenzymatic analysis"**, register number 017/2010, of Renato Corrêa Viana Casarin, Hugo Felipe do Vale, Márcio Zaffallon Casati and Tiago Taiete, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 10/30/2010.

Prof. Dr. Pablo Agustin Vargas
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Prof. Dr. Jacks Jorge Junior
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CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
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