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Silvia Cardoso De David

CORRELAÇÕES ENTRE BIOFILME E CONDIÇÃO GENGIVAL EM DIFERENTES FREQUÊNCIAS DE HIGIENE BUCAL

Santa Maria, RS
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Dissertação apresentada ao Curso de Mestrado do Programa de Pós- Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, Ênfase em Periodontia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Ciências Odontológicas**.

Orientador: Prof. Dr. Carlos Heitor Cunha Moreira

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Aprovado em 26 de julho de 2016:

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Carlos Heitor Cunha Moreira, Dr.
(Presidente/Orientador - UFSM)

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Tiago Fiorini, Dr. (UFRGS)

_____

José Mariano da Rocha, Dr. (UFSM)

Santa Maria, RS
2016

DEDICATÓRIA

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RESUMO

CORRELAÇÕES ENTRE BIOFILME E CONDIÇÃO GENGIVAL EM DIFERENTES FREQUÊNCIAS DE HIGIENE BUCAL

AUTORA: SILVIA CARDOSO DE DAVID
ORIENTADOR: CARLOS HEITOR CUNHA MOREIRA

A gengivite é considerada a forma mais prevalente dentre as doenças periodontais. Evidências têm mostrado a importância da gengivite como precursora da perda de inserção periodontal e perda dental. A despeito de sua importância, muitas vezes o diagnóstico da inflamação gengival é negligenciado. Para a manutenção de saúde gengival, procedimentos de desorganização mecânica do biofilme devem ser realizados de forma efetiva e periódica. Estudos observaram que frequências de higiene bucal (HB) de 12 e 24 horas estão relacionadas a manutenção de saúde gengival. Tão importante quanto a frequência, é a qualidade em que esse procedimento é realizado. Estudos que avaliam a associação entre frequência de escovação, desorganização efetiva do biofilme e alterações clínicas gengivais têm um papel importante na prevenção e tratamento das gengivites. Portanto, o objetivo deste estudo foi avaliar a existência de correlação entre biofilme e inflamação gengival em indivíduos que realizaram efetivo controle do biofilme em diferentes frequências. Os dados deste estudo foram coletados de um ensaio clínico randomizado realizado no ano de 2012 na cidade de Santa Maria, RS, Brasil. A amostra foi composta por 52 estudantes de cursos não relacionados a área da saúde da UFSM inicialmente randomizados em 4 frequências de higiene bucal e agrupados em 2: grupo G12/24 (12 e 24h) e grupo G48/72 (48 e 72h). No G12/24, mesmo havendo aumento nos níveis de Índice de Placa (IPl) durante os 30 dias, os níveis de inflamação gengival não diferiram estatisticamente do *baseline*. Já no G48/72, aumento nos níveis de IPl foi acompanhado por aumento nos níveis de inflamação gengival. Houve correlação positiva em ambos os grupos, no entanto, a correlação diminuiu no G12/24, demonstrando que o aumento nos níveis de biofilme não foi suficiente para desenvolver resposta inflamatória gengival. Em indivíduos que realizam controle efetivo do biofilme, a correlação entre biofilme e inflamação gengival é afetada pela frequência de higiene bucal.

Palavras-chave: Biofilme. Gengivite. Inflamação gengival.

ABSTRACT

CORRELATIONS BETWEEN BIOFILM AND GINGIVAL STATUS AT DIFFERENT ORAL HYGIENE FREQUENCIES

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Gingivitis is the most prevalent form among periodontal diseases. Evidence has shown the importance of gingivitis as a precursor of periodontal attachment loss and tooth loss. Despite its importance, often the diagnosis of gingival inflammation is overlooked. For maintaining gingival health, biofilm mechanical removal procedures should be perfomed effectively and periodically. Studies have shown oral hygiene frequencies of 12 and 24h are related to the gingival health maintenance. As important as frequency, it is the quality in which the procedure is performed. Studies assessing the association between toothbrushing frequency, effective biofilm removal and gingival clinical changes have an important role in the prevention and treatment of gingivitis. The aim of this study was to evaluate correlation between plaque and gingival inflammation in individuals underwent effective biofilm control at different frequencies. Data from this study were collected from a randomized clinical trial conducted in 2012 in Santa Maria, RS, Brazil. The sample consisted of 52 UFSM students, of courses not related to health, initially randomized into 4 oral hygiene frequencies and grouped into 2: Group G12/24 (12 and 24h) and group G48/72 (48 and 72h). In G12/24, even with an increase in plaque levels during 30 days, gingival inflammation levels were not statistically different from baseline. In G48/72, increase in plaque levels were accompanied by increasing levels of gingival inflammation. Positive correlation was observed in both groups, however, correlation is reduced in G12/24, demonstrating that increase in plaque levels were not sufficient to develop inflammatory response. In subjects who perform effective biofilm control, the correlation between plaque and gingival inflammation is affected by oral hygiene frequency.

Keywords: Biofilm. Gingivitis. Gingival Inflammation.

SUMÁRIO

1 INTRODUÇÃO GERAL

Gengivite induzida por placa é a inflamação nos tecidos periodontais de proteção causada pelo acúmulo de bactérias na margem gengival (MARIOTTI, 1999). A gengivite é considerada a forma mais prevalente dentre as doenças periodontais (ABABNEH et al., 2012; IDREES et al., 2014; OPPERMANN et al., 2015). Albandar (2002) verificou que 82% dos adultos norte-americanos de 19 anos ou mais apresentam gengivite em pelo menos um dente. No Brasil, Chiapinotto e colaboradores (2013) constataram que 78,2% dos escolares de 8 a 12 anos têm gengivite. Estes dados sugerem que a doença é prevalente tanto em países desenvolvidos como em desenvolvimento e presente em várias faixas etárias. Os sinais clínicos compreendem alterações de cor e consistência, edema e sangramento gengival espontâneo ou após estímulo (MARIOTTI, 1999). Essas características são confinadas ao tecido gengival e completamente reversíveis com a remoção dos fatores etiológicos (LOE; THEILADE; JENSEN, 1965). O diagnóstico da gengivite pode ser realizado em extensão e gravidade e existem vários índices com este intuito, como o índice de sangramento gengival (EDWARDS, 1975), o índice de sangramento sulcular (MUHLEMANN; SON, 1971) e o índice gengival (LOE, 1967). Estes índices consideram uma ou mais das seguintes alterações: cor e contorno da gengiva, sangramento gengival, extensão do envolvimento gengival e fluido crevicular gengival (CIANCIO, 1986). A maioria deles utiliza escores para facilitar seu uso em estudos epidemiológicos.

Os sinais clínicos da gengivite podem variar entre indivíduos, dentes e sítios. Fatores como má-nutrição (DIETRICH et al., 2005), alterações hormonais associadas à gestação e período pré-menstrual (WU et al., 2015; BORGO et al., 2014; MACHTEI et al., 2004), diabetes (CARNEIRO et al., 2015), fumo (BERGSTROM, 1990; GIANNOPOULOU et al., 2003) e algumas medicações (BRUNET et al., 2001) podem alterar a expressão clínica da inflamação gengival. A gengivite pode favorecer a colonização subgengival dos biofilmes e, em indivíduos suscetíveis, pode levar a perda de inserção (ADDY; ADRIAENS, 1998). Algumas evidências têm demonstrado a importância da gengivite como precursora da perda de tecidos de suporte periodontal e da perda dentária (SCHATZLE et al., 2003; LANG et al., 2009). Schatzle e colaboradores (2003) observaram que pacientes que mantiveram saúde gengival, em 26 anos, apresentaram menos do que 2 mm de

média de perda de inserção cumulativa, enquanto sítios com sangramento gengival (escore 2 do IG) apresentaram uma média de perda de inserção cumulativa maior do que 3 mm. Além disso, dentes associados com gengivite apresentaram risco maior para perda dentária do que aqueles associados com gengiva saudável ou levemente inflamada (LANG et al., 2009). O estabelecimento longitudinal de adequado autocontrole de biofilme resulta não somente na prevenção da gengivite, mas também da perda de inserção periodontal (HUGOSON et al., 2008). Apesar de sua importância, diagnóstico e tratamento da gengivite são, muitas vezes, negligenciados.

A desorganização mecânica do biofilme é considerada o padrão-ouro tanto para a prevenção quanto para o tratamento da gengivite. A frequência na qual os biofilmes são desorganizados foi avaliada em um estudo clássico em 1973 (LANG et al., 1973). Frequências de até 48h foram compatíveis com saúde gengival e indivíduos com frequências de higiene bucal (HB) de 72 e 96h desenvolveram gengivite. Recentemente dois estudos avaliaram a frequência de HB e as alterações gengivais com uso de dentifrícios com agentes antimicrobianos (PINTO et al., 2013) ou não (de FREITAS et al., 2016), a frequência de escovação compatível com saúde gengival foi de até 24h. Frequências de 48 e 72h resultaram em aumento dos níveis de inflamação gengival.

A qualidade da HB é um fator determinante para a manutenção de saúde gengival. Apesar de indivíduos relatarem frequências adequadas de HB, a maioria não a realiza com a qualidade necessária para prevenir acúmulo de biofilme (JEPSEN, 1998), o que é confirmado pela alta prevalência de gengivite (MAYFIELD; ATTSTROM; SODERHOLM, 1998, ADDY; ADRIAENS, 1998). Para manutenção da saúde gengival é necessário desorganizar o biofilme de forma periódica e efetiva, afim de não ocorrer amadurecimento do mesmo. Theilade e colaboradores (1966) e Teles e colaboradores (2012) observaram um padrão de sucessão bacteriana durante a maturação do biofilme. Bactérias compatíveis com saúde e em equilíbrio são sucedidas por bactérias mais patogênicas a medida que ocorre o aumento na quantidade de biofilme (HAFFAJEE et al., 2009). Correlações e associações entre alterações microbiológicas do biofilme e condição gengival (THEILADE et al., 1966, HAFFAJEE et al., 2009) bem como correlação positiva entre quantidade de biofilme e inflamação gengival (OLIVEIRA et al., 2015, LIE et al., 1998) estão sedimentadas na literatura. Além disso, a HB de forma adequada resulta em melhor condição

gengival (HUGOSON et al., 1998). De acordo com o nosso conhecimento, há ausência de informações a respeito da associação entre frequências de escovação, desorganização efetiva do biofilme e alterações clínicas gengivais. Portanto, ressalta-se a importância de avaliar a condição gengival frente ao acúmulo de biofilme em pacientes que realizam HB, com qualidade, em diferentes frequências.

2 ARTIGO

CORRELATION BETWEEN PLAQUE CONTROL AND GINGIVAL HEALTH USING SHORT AND EXTENDED ORAL HYGIENE INTERVALS

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Running tittle: Plaque control and gingival health

Key words: dental plaque; periodontal diseases; gingivitis; oral hygiene, tooth brushing

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ABSTRACT

Aim: To evaluate correlation between dental plaque formation and gingival health in subjects performing high standard oral hygiene at short and extended intervals.

Methods: Fifty-two non-dental students volunteered for this study. The subjects, trained to perform high oral hygiene standards, were randomized to perform oral hygiene at 12, 24, 48 or 72h intervals over 30 days. The Plaque Index (PlI) and the Gingival Index (GI) were evaluated at baseline, 15 and 30 days. For the statistical analysis oral hygiene intervals were collapsed into daily (G12/24; 12 and 24h) and extended (G48/72; 48 and 72h) intervals. Summary statistics (mean±SD) and Spearman correlations between the PlI and the GI at baseline, 15 and 30 days were estimated.

Results: At baseline, correlation coefficients between PlI and GI were positive for both groups ($r=0.29$ and $r=0.25$). At day 15 and 30, correlation was maintained with similar baseline values for the G48/72 group. GI levels did not increase despite an increase in PlI for the G12/24 group, and the correlation was lower than that observed at baseline ($r=0.13$ vs. $r=0.29$).

Conclusion: In subjects with high oral hygiene standards, the oral hygiene frequency governs the correlation between dental plaque formation and gingival health. Subjects performing high oral hygiene standards at daily intervals will maintain gingival health in difference to subjects using extended hygiene intervals.

CLINICAL RELEVANCE

Scientific rationale for study: The correlation between dental plaque accumulation and gingival health is already established. However, the correlation between high standard self-performed oral hygiene at different intervals and gingival health is not yet completely determined.

Principal findings*:* In subjects with high oral hygiene standards, the oral hygiene frequency governs the correlation between dental plaque formation and gingival health.

Practical implications: Subjects performing high oral hygiene standards at daily intervals will maintain gingival health in difference to subjects using extended hygiene intervals.

INTRODUCTION

Gingivitis is a reversible inflammatory condition induced by persistent microbial dental plaque formation (Mariotti 1999). High prevalence has been observed in both developed and developing countries encompassing up to 100% of the populations (Albandar 2002, Ababneh *et al.* 2012, Chiapinotto *et al.* 2013, Idrees *et al.* 2014, Oppermann *et al.* 2015). Moreover, gingivitis has been associated with increased risk for attachment loss, halitosis, and poor self-awareness (Schätzle *et al.* 2003, Pham *et al.* 2012, Tomazoni *et al.* 2014). The pivotal study by Løe *et al.* (1965) established the causal relationship between dental plaque and gingivitis. Subsequent studies in support demonstrated a correlation between dental plaque and gingivitis (Breuer & Cosgrove 1989, Lie *et al.* 1998, Oliveira *et al.* 2015).

Regularly performed oral hygiene measures using tooth brushing and interproximal approaches represent the major form of gingivitis prevention and resolution (Kistler *et al.* 2013). Nevertheless, many individuals do not, or cannot, perform satisfactory oral hygiene measures to control dental plaque formation and thus prevent gingival inflammation (Claydon 2008). Frequency of oral hygiene has been associated with gingival health maintenance. Recent studies evaluated gingival health over 30 days following two different oral hygiene regimes. Subjects that performed oral hygiene at 12 and 24h intervals maintained gingival health. However, gingival health deteriorated when oral hygiene frequencies were reduced to 48 and 72h intervals (Pinto *et al.* 2013, de Freitas *et al.* 2016).

Alterations in composition and quantity of dental plaque and changes in gingival health are associated with the quality and frequency oral hygiene measures (Claydon 2008, Theilade *et al.* 1966). Assessments of oral hygiene quality and frequency are routinely performed at clinical practice to establish a need for patient behavior modification; effective oral hygiene routines directly related to a lower percentage of sites with dental plaque. Assessments of gingival health reveals whether the patient's oral hygiene periodicity is adequate further indicating directions of any required habitual intervention (Pinto *et al.* 2013).

Despite the correlation between dental plaque formation and gingivitis is well established, its magnitude can decrease when regular effective oral hygiene measures are performed. The association between oral hygiene frequency, high standard self-performed oral hygiene measures, and gingival health has not been directly evaluated. This study aimed to evaluate the correlation between dental plaque formation and gingival health in individuals with high standard self-performed oral hygiene measures at different intervals. We hypothesize a weaker correlation between dental plaque formation and gingival health in the presence of appropriate frequency and quality of personal oral hygiene.

MATERIAL AND METHODS

Study Design

This study represents a secondary analysis of a randomized clinical trial elaborated between February and October 2012 at the Postgraduate Periodontics Clinic, Federal University of Santa Maria (de Freitas *et al.* 2016). The original study methodology is described in brief.

Sample

Non-dental students from the Federal University of Santa Maria, minimum age 18 years, were considered eligible. Subjects should present papilla completely filling the interdental space, maximum 15% of sites exhibiting gingival bleeding (GI=2; Løe 1967) and absence of interproximal attachment loss. Subjects exhibiting periodontitis, xerostomia, diabetes mellitus, psychomotor disturbances, pregnancy, smoking habit, orthodontic appliances and fixtures, or having used anti-inflammatory or antibiotic medications within previous three months were excluded from participating in the study.

Pre-experimental period, randomization and experimental period

Two periodontists (DAMD and GCF) provided individualized oral hygiene instructions for all subjects using toothbrush and dental floss. Instructions were repeated weekly in order to obtain effective plaque control considered optimal when ≤5% of the sites exhibited GI scores 2 or 3 (Løe 1967). When patients met this criteria, they received coronal polishing and were randomized to perform oral hygiene at 12, 24, 48 or 72h intervals. Allocation sequence was generated using a computer program (Random Allocation Software, version 1.0) and maintained confidential using opaque envelopes. All subjects received soft a multi-bristle toothbrush (Oral-B® Indicator® Plus, size 30, Gross-Gerdau, Germany), dental floss (Oral-B® Essential Floss Tarpaulin, Gross-Gerau, Germany) and dentifrice (Oral-B® 1.2.3, Gross-Gerau, Germany). Dentifrice quantity was standardized (a single point across the brush, approximately 0.5g) and flossing performed only in conjunction with tooth brushing. Subjects received a schedule and were reminded on days they should not perform oral hygiene by a telephone call. At end of study, patients were instructed to return their habitual oral hygiene measures and dentifrice tubes were collected and weighed to assess compliance (Digital Balance Scale Professional-Mini, model 1480, Tania Corp, Japan).

Clinical Parameters

The Plaque Index (PlI; Silness & Løe 1964) and the Gingival Index (GI; Løe 1967) were assessed at six sites per tooth excluding third molars at baseline, 15 and 30 days. An experienced clinician (CHCM) trained two dentists to assess the PlI (DAMD) and the GI (GCF). Examiners were masked relative to experimental groups. After the PlI assessment, subjects were instructed to perform oral hygiene in order to mask the second examiner relative to dental plaque accumulation when assessing the GI.

Ethical considerations

After an explanation of the purpose of study and any questions answered by one of the investigators, subjects agreeing to participate in this study signed an Informed Consent. This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee in Research of Federal University of Santa Maria. (CAAE: 0186.0.243.00-10).

Statistical Analysis

For the statistical analysis, oral hygiene intervals of 12 and 24, and 48 and 72h were collapsed into two groups, G12/24 and G48/72, respectively. The Spearman correlation coefficient between PlI and GI for both groups was calculated at baseline, 15 and 30 days. Mean (±SD) PlI and GI was calculated to verify clinical parameters behavior relative to oral hygiene intervals. Intragroup differences were determined using repeated measures ANOVA. Intergroup differences at baseline were verified using a chi-square test and independent t-test. Statistical analysis was performed using a statistical software (SPSS, version 21.0, Chicago, IL, USA). Significance level was set at 5%. Primary outcome of the study was correlation between PlI and GI.

RESULTS

Table 1 shows group demographics and clinical parameters at baseline. Groups did not differ statistically relative to age and gender, and presented with shallow probing pocket depths and minimal clinical attachment loss.

Statistically significant increases in mean GI for the G12/24 group did not manifest ($p=0.52$) despite mean PlI significantly increased from baseline through day 15 to maintain this level through day 30 ($p<0.0001$). In contrast, mean PlI ($p<0.0001$) and mean GI ($p<0.0001$) for the G48/72 group increased from the baseline though day 15 and 30 (Table 2).

Positive, statistically significant correlations were observed between PlI and GI means at baseline for the G12/24 and G48/72 groups, (r=0.29 and r=0.25, respectively). PlI/GI correlations decreased for the G12/24 group day 15 (r=0.15) and day 30 (0.13). In contrast, PlI/GI correlations maintained over the study period for the G48/72 group, day 15 (r=0.21) and day 30 (r=0.23) (Table 3).

Figure 1 shows fluctuations in PlI and GI scores over the course of study (baseline, 15 and 30 days). Percentage of sites with PlI score 1 and 2 increased, and a reduction in sites with score 0 were observed for the G12/24 group, while minimal alterations in percentage sites with GI score 0, 1 and 2 were observed. In contrast, for the G48/72 group, an increase in PlI 1 and 2 scores was accompanied by an increase in GI 1 and 2 scores.

DISCUSSION

This study evaluated the correlation between dental plaque formation and gingival health in subjects with gingival health who performed high standard oral hygiene at 12, 24, 48 or 72h intervals over 30 days. The correlation between PlI and GI was maintained only in the G48/72 extended oral hygiene interval group in which an increase in dental plaque formation was accompanied by increase in gingival inflammation. In the daily, G12/24 oral hygiene interval group, although the PlI average increased, GI means maintained and the correlation coefficient decreased compared with baseline. Thus, an increase in dental plaque formation was not sufficient to modify the initial gingival condition in individuals who performed oral hygiene daily every 12 or 24h. These results corroborate other recent studies that assessed necessary oral hygiene frequencies to maintain gingival health (Pinto *et al.* 2013; de Freitas *et al.* 2016). Regardless of mechanical plaque control with or without antimicrobial dentifrices, GI means remained unaltered in health at oral hygiene intervals up to 24h. Individuals who performed oral hygiene at longer intervals showed increase in GI means.

The G12/24 and G48/72 groups showed different patterns in distribution of PlI and GI scores. The G12/24 group showed increase in PlI 1 and 2 scores without alterations in GI scores, while both PlI and GI scores increased in the G48/72 group. These results contradict Haffajee *et al.* (2009) who observed positive association between an increase in plaque mass and gingival redness (GI 1) and marginal gingival bleeding (GI 2). Absence of GI alterations in G12/24 group in the present study suggest that daily hygiene intervals did not allow sufficient time for microbial succession, the microbiota changing within 2 days of oral hygiene abstention, within 4-9 days mature microbial colonization provoking gingival inflammation (Theilade *et al.* 1966). Thus, gingival health was maintained in the G12/24 group despite increased PlI scores, dental plaque formation/maturation effectively disrupted every 12 or 24h providing dental plaque formation compatible with gingival health. In G48/72 group, however, extended oral hygiene intervals may allowed more complex bacterial colonization (Heller *et al.* 2016). As bacterial colonization is not subject to early/frequent disruption, qualitative and quantitative changes favor pathogenic species (Haffajee *et al.* 2009, Teles *et al.* 2012) initiating a clinical gingival inflammatory response.

Mature microbial dental plaques persisting over extended periods of time without disruption are not compatible with gingival health (Theilade et al. 1966). However, effective periodic disruption (12h and 24h frequency) is sufficient to avoid dental plaque maturation and consequently to maintain gingival health. Eligibility criteria for the present study required subjects with healthy gingival conditions, which were obtained selecting subjects with high oral hygiene standards. Here, the results confirm that the quality of dental plaque disruption should be associated with oral hygiene periodicity, and these characteristics have the same importance.

In summary, in the evaluation of subjects with high oral hygiene standards presenting with gingival health following a closely monitored structured protocol we conclude that in individuals who perform effective plaque control, the correlation between dental plaque formation and gingival health is affected by the oral hygiene frequency. Subjects performing high

oral hygiene standards at daily intervals will maintain gingival health in difference to subjects using extended hygiene intervals.

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TABLES

Table 1 – Group demographics and clinical parameters at baseline (mean±SD).

	G12/24 (n=26)	G48/72 (n=26)
Age (years)	23.3±2.9	24.0±3.1
Gender		
Female n (%)	12 (46)	16 (62)
Male n (%)	14 (54)	10 (38)
Probing pocket depth (mm)	1.8±0.2	1.8±0.3
Clinical attachment level (mm)	0.02 ±0.05	0.00±0.01

No statistical differences were observed among experimental groups at baseline.

Table 2 – Mean (±SD) group PlI and GI at baseline, 15 and 30 days.

	PlI				GI			
	Baseline	**15 days**	**30 days**	**p***	**Baseline**	**15 days**	**30 days**	**p***
G12/24	0.15±0.06	0.36±0.19	0.35±0.23	0.00	0.50±0.10	0.48±0.09	0.50±0.11	**0.52**
G48/72	0.14±0.07	0.75±0.31	0.66±0.26	0.00	0.51±0.11	0.83±0.11	0.88±0.10	0.00

*Repeated measures ANOVA

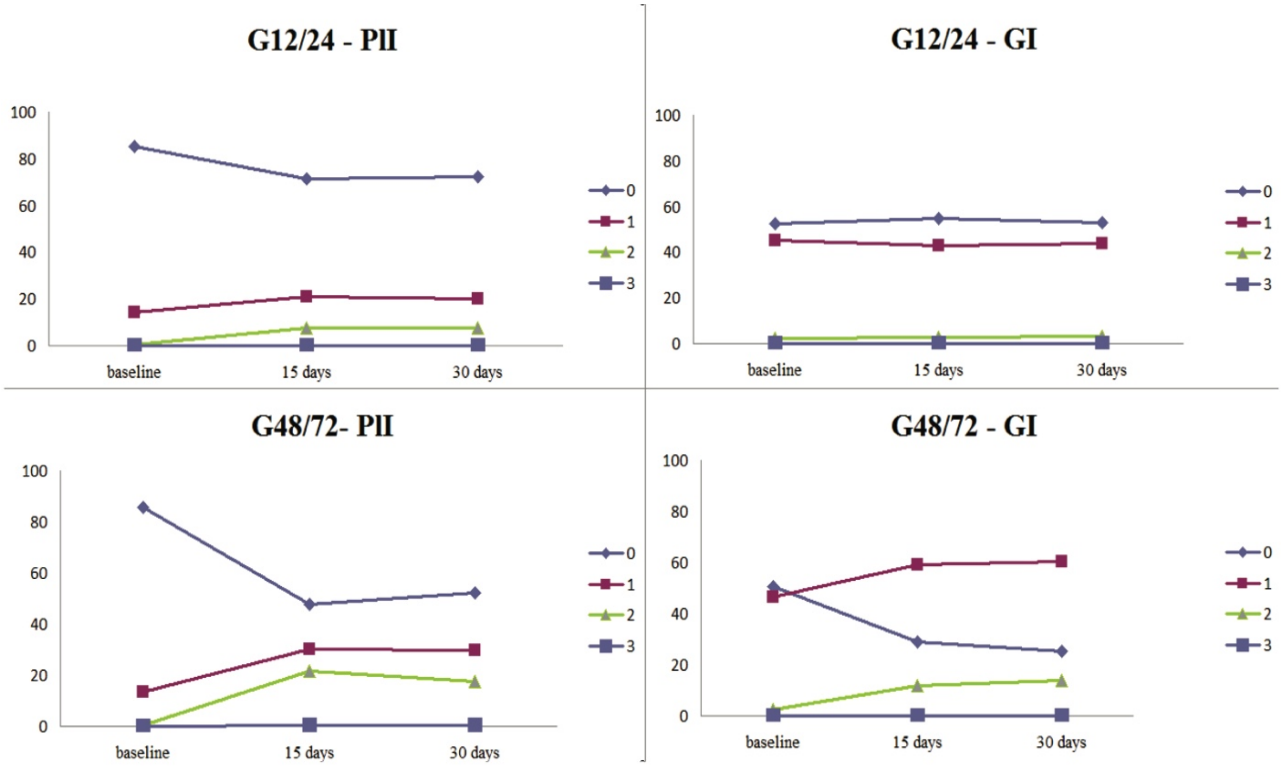
Table 3 – Correlation between PlI and GI according to experimental group at baseline, 15 and 30 days.

	Baseline	15 days	30 days
G12/24	0.29*	0.15*	0.13*
G48/72	0.25*	0.21*	0.23*

*Spearman correlation coefficient ($p<0.0001$)

FIGURES

Figure 1 – Frequency (%) PlI and GI scores by oral hygiene interval, every 12/24 and 48/72h.



3 CONCLUSÃO

Este estudo avaliou correlação entre biofilme e inflamação gengival em indivíduos que realizaram HB efetiva em diferentes frequências. Nossos resultados demonstraram que os valores de correlação foram mantidos para o grupo com maiores intervalos de HB (G48/72) e diminuíram para o G12/24, no qual o aumento nas médias de biofilme não foi suficiente para aumentar as médias de inflamação gengival. Assim, em pacientes saudáveis que desorganizam o biofilme de forma efetiva, a força de correlação é afetada pela frequência de higiene bucal.

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