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Effectiveness of Non-Surgical Treatment to Reduce IL-18 Levels in the Gingival Crevicular Fluid of Patients with Periodontal Disease

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The aim of this study was to evaluate the effectiveness of the non-surgical periodontal treatment in reducing the gingival crevicular fluid (GCF) levels of IL-18 from inflamed periodontal sites. Fourteen patients with periodontal disease were included, being 9 patients with chronic periodontitis (mean age: 48.8 SD ± 7.4 years) and 5 patients with gingivitis (mean age: 43.6 SD ± 11.8). The patients were divided in the following groups: gingivitis sites from periodontitis patients (sites GP), periodontitis sites from periodontitis patients (sites PP), and gingivitis sites from gingivitis patients (sites GG). Probing pocket depth (PPD), probing attachment level (AL), plaque index (PI) and gingival index (GI) were recorded, and gingival fluid samples were collected. The subjects received non-surgical treatment and were re-evaluated 30 days after treatment (day 30 AT). There was a significant reduction in PI in GG (1.0 ± 0.4 to 0.5 ± 0.2), GP (1.2 ± 0.3 to 0.5 ± 0.3), and in PP (1.3 ± 0.4 to 0.7 ± 0.3) 30 AT. There was also a significant reduction in the GI in GG (1.3 ± 0.3 to 0.7 ± 0.4). PPD reduced significantly in GG (2.4 ± 0.6 to 1.9 ± 0.1), and PP (6.7 ± 1.1 to 5.2 ± 0.9) 30 AT. When all the samples were analyzed together, there was a significant reduction in IL-18 (12.9 ± 7.2 to 10.0 ± 3.1). This study showed that non-surgical treatment was effective in reducing GCF levels of IL-18 from inflamed periodontal sites.

Key Words: Interleukin-18, periodontitis, periodontal treatment.

INTRODUCTION

Periodontal disease progression is due to a combination of factors including the presence of periodontopathic bacteria and high levels of proinflammatory cytokines, such as interleukin (IL)-1b (1), tumor necrosis factor (TNF)-α (2) and more recently IL-18 (3).

IL-18 is a proinflammatory cytokine related to the IL-1 family that is produced by Kupffer cells, activated macrophages, keratinocytes, intestinal epithelial cells, osteoblasts and adrenal cortex cells (4). The primary functions of IL-18 include the induction of IFN-g and TNF-α in T cells and natural Killer (NK) cells (5), up-regulation of Th1 cytokines including IL-2, granulocyte macrophage colony stimulating factor and IFN-g (4). It plays an important role in the innate immunity and it has been shown to induce not only T helper (Th) 1 but also Th2 cytokines, such as IL-4, IL-5, IL-10 and IL-13 (6). IL-18 is described to be upregulated in different chronic diseases, including type I diabetes, lupus and Crohn's disease (7).

Johnson and Serio (8) and Pradeep et al. (9) showed a highly significant correlation between IL-18 concentration and pocket depth, suggesting that IL-18 could be a useful target for periodontal therapy. A higher salivary concentration of IL-18 in patients with chronic periodontitis compared with healthy individuals has been demonstrated (10), suggesting that an elevated salivary IL-18 level in untreated chronic periodontitis patients has the potential to be a biomarker for periodontal tissue destruction. Orozco et al. (3) observed that IL-18 was

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higher in gingivitis sites from periodontitis patients when compared with gingivitis sites from control patients.

Recently, our research group demonstrated that IL-18 in the gingival crevicular fluid (GCF) is higher in periodontitis patients and is associated with periodontopathogens from the red complex (11). Therefore, it is reasonable to believe that IL-18 might be involved in periodontal destruction and progression, making it interesting the analysis of the local effect of periodontal treatment. Thus, the aim of this study was to evaluate the effectiveness of the non-surgical periodontal treatment in reducing the GCF levels of IL-18 from inflamed periodontal sites.

MATERIAL AND METHODS

Fourteen subjects participated in this study. Nine patients (mean age: 48.8 SD ± 7.4 years) with generalized chronic periodontitis and 5 gingivitis controls subjects (mean age: 43.6 SD ± 11.8 years) who were attending the Dental School of the State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil. Pedro Ernesto University Hospital's Ethics Committee (UERJ, Rio de Janeiro, Brazil) approved this study. The participants had no ongoing systemic disease or infections and gave written informed consent to participate.

The clinical measurements were taken at 6 sites *per* tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) of every tooth present, except for the third molars, with a Williams probe (PCP10 Color Coded Probe; Hu-Friedy Co., Chicago, IL, USA), by the same calibrated examiner. The parameters measured were: the plaque index (12); (PI), gingival index (13) (GI), probing pocket depth (PPD) and clinical attachment level (AL).

GCF was collected with an intracrevicular washing device modified from Salonen and Paunio (14). The sites to be sampled were isolated with cotton rolls and dried gently with an air syringe. Supragingival plaque was carefully removed before sampling. Each pocket selected was washed 5 times with 5 mL of phosphate buffered saline (PBS) during continuous aspiration. Samples from the same type of site in each person were pooled together, diluted with PBS up to 1 mL and immediately centrifuged at 3,000 x g for 10 min. The supernatant was collected and frozen at -20ºC, until analysis.

In the patient group, samples were taken from 5 sites with pocket depth ≥5 mm and GI 1-2 (PP - periodontitis sites from periodontitis patients) and 5 sites with pocket depth ≤3 mm and GI 1-2 (GP - gingivitis sites from periodontitis patients). In the control group, 5 sites with pocket depth ≤3 mm with GI 1-2 (GG - gingivitis sites from gingivitis patients) were sampled.

IL-18 was measured in GCF using a commercially available ELISA kit (Quantikine HS; R&D Systems, Minneapolis, MN, USA and MBL, Nagoya, Japan), following the respective manufacturer's instructions. IL-18 amounts were expressed as picograms (pg).

The patients with periodontitis received non-surgical periodontal treatment, which comprised instructions about oral hygiene and supra- and subgingival debridement (scaling and root planing) under local anaesthesia. The treatment took, on average, 4 sessions of 40 min, within 2 weeks. The control group received the same treatment except for the subgingival scaling. The scaling and root planing was performed with manual instruments (Gracey and McCall Curettes; Hirschfield Files, HU-Friedys Mfg. Co. Inc.) and by a single trained operator. Re-evaluation was performed 30 days after completion of non-surgical treatment.

The significance of the differences between day 0 and 30 AT, as well as between GP and PP sites, was calculated with the Wilcoxon signed-rank test. The significance of the differences between gingivitis and periodontitis patients was calculated with the Mann-Whitney U test. Probability values higher than 0.05 were considered as not significant.

RESULTS

There was a statistically significant reduction ($p<0.05$) in the PI of GG from 1.0 ± 0.4 to 0.5 ± 0.2, PI of GP from 1.2 ± 0.3 to 0.5 ± 0.3, PI of PP from 1.3 ± 0.4 to 0.7 ± 0.3 30 AT. There was also a significant reduction ($p<0.05$) in the GI of GG from 1.3 ± 0.3 to 0.7 ± 0.4. PPD reduced significantly in GG from 2.4 ± 0.6 to 1.9 ± 0.1, and in PP from 6.7 ± 1.1 to 5.2 ± 0.9 in day 30 AT (Table 1).

When all the samples (GG, GP and PP) were analyzed together, there was a statistically significant reduction ($p<0.05$) in PI from 1.2 ± 0.4 to 0.6 ± 0.3, GI from 1.1 ± 0.4 to 0.8 ± 0.5 and PPD from 4.1 ± 2.2 to 3.2 ± 1.6. In levels of IL-18, there was also a significant reduction ($p<0.05$) from 12.9 ± 7.2 to 10.0 ± 3.1 (Table 2).

The clinical parameters (PI, GI, PPD, AL) and the total amounts of IL-18 from each patients are presented

in Table 3.

DISCUSSION

The present study revealed a significant reduction in the GCF levels in inflamed periodontal sites after nonsurgical periodontal treatment. To the best of our knowledge, there is only one study evaluating the effectiveness of the periodontal treatment in the levels of IL-18 (9). The effect of periodontal treatment has been investigated in others pro-inflammatory cytokines. Gamonal et al. (15) showed that the periodontal treatment reduced the levels of IL-1b, IL-8 in the GCF. Goutoudi et al. (16) also observed a reduction of the total amount of IL-1b after periodontal therapy.

IL-18 levels in GCF from inflamed periodontal disease sites have been investigated. Johnson and Serio (8) demonstrated that IL-18 concentration was higher in gingival biopsies adjacent to sites where the probing depth was >6 mm when compared to healthy sites. Pradeep et al. (9) showed a similar result, but in GCF, where the levels of IL-18 were higher before treatment and decreased after non-surgical treatment. When the groups were analyzed separately, the levels of IL-18 were higher in patients with gingivitis and in the deepest pockets of patients with periodontitis. We believe that this might have occurred due to the small number of patients in the study.

Orozco et al. (3) found IL-18 to be increased in shallow inflamed sites in periodontitis patients when compared with gingivitis sites in control patients. Our research group obtained similar results, but we also found that the red complex was significantly increased in the PP sites when compared with the GG and GP sites, thus suggesting the existence of an association between the presence of red complex species and higher levels of IL-18 (11).

In general, non surgical therapy was effective to control the progression of periodontal disease. However, attachment loss after treatment and during maintenance may occur in the absence of signs of chronic inflammatory periodontal disease (17). It is reasonable to believe that pro-inflammatory cytokines might be involved in the subclinical activation of the periodontal inflammation. We understand that there were certain limitations to the current study including the limited number of subjects and samples evaluated, but this pilot study will encourage further investigations about the importance of the IL-18 in the disease progression.

In conclusion, this study showed that non-surgical treatment is effective in reducing GCF levels of IL-18 from inflamed periodontal sites.

Table 1. Mean values (±SD) for plaque index (PI), gingival index (GI), probing pocket depth (PPD), attachment level (AL) and total amounts of IL-18 (IL-18) expressed in picograms (pg) in 5 gingivitis sites from subjects with gingivitis alone (GG), and in 9 gingivitis sites (GP) and 9 periodontitis sites (PP) from patients with chronic generalized periodontitis.

	Before treatment			After treatment		
	GG (=5)	GP (n=9)	PP (n=9)	GG (n=5)	GP (n=7)	PP (n=7)
PI	1.0 ± 0.4	1.2 ± 0.3	1.3 ± 0.4	0.5 ± 0.2$^{\#}$	0.5 ± 0.3$^{\#}$	0.7 ± 0.3$^{\#}$
GI	1.3 ± 0.3	1.1 ± 0.4	1.0 ± 0.4	0.7 ± 0.4$^{\#}$	0.8 ± 0.6	0.8 ± 0.5
PPD	2.4 ± 0.6	2.7 ± 0.6	6.7 ± 1.1$^{\Sigma\S}$	1.9 ± 0.1$^{\#}$	2.3 ± 0.4	5.2 ± 0.9$^{\#\Sigma\S}$
AL	0.5 ± 1.0	1.1 ± 0.8	7.8 ± 1.5$^{\Sigma\S}$	0.6 ± 1.0	1.3 ± 0.9	7.2 ± 1.3$^{\Sigma\S}$
IL-18	15.0 ± 8.3	9.3 ± 3.4	15.3 ± 8.4	11.5 ± 2.1	8.0 ± 3.1	11.0 ± 3.0

$^{\#}$Significance of the difference before and after treatment; $p<0.05$. §Significance of the difference between GP and PP. $^{\Sigma}$Significance of the difference between GG and PP.

Table 2. Mean values (±SD) for plaque index (PI), gingival index (GI), probing pocket depth (PPD), attachment level (AL) and total amounts of IL-18 (IL-18) expressed in picograms (pg) in all groups together.

	Before treatment (n=23)	After treatment (n=19)
PI	1.2 ± 0.4	0.6 ± 0.3$^{\#}$
GI	1.1 ± 0.4	0.8 ± 0.5$^{\#}$
PPD	4.1 ± 2.2	3.2 ± 1.6$^{\#}$
AL	3.5 ± 3.6	3.1 ± 3.2
IL-18	12.9 ± 7.2	10.0 ± 3.1$^{\#}$

$^{\#}$Significance of the difference before and after treatment; $p<0.05$.

RESUMO

O objetivo desse estudo foi avaliar o efeito do tratamento periodontal não-cirúrgico sobre os níveis da IL-18 em sítios inflamados de pacientes com doença periodontal. Foram avaliados 14 pacientes com doença periodontal, sendo 9 pacientes com periodontite crônica generalizada (idade média: 48,8 DP ± 7,4 anos) e 5 pacientes com gengivite (idade média: 43,6 DP ± 11,8 anos). Os pacientes foram divididos nos seguintes grupos: sítios sem perda de inserção nos pacientes com periodontite (GP), sítios com perda de inserção nos pacientes com periodontite (PP) e sítios sem perda de inserção nos pacientes com gengivite (GG). Profundidade de bolsa (PB), nível de inserção (NI), índice de placa (IP) e índice gengival (IG) foram avaliados e amostras do fluido gengival foram coletadas. Os pacientes receberam terapia não-cirúrgica e foram reavaliados 30 dias após tratamento (AT). Houve uma redução significante no IP dos GG (1,0 ± 0,4 para

Table 3. Individuals values for Plaque Index (PI), Gingival index (GI), probing pocket depth (PPD), attachment level (AL) and total amounts of IL-18 (IL-18) expressed in picograms (pg) in and from 9 sites from gingivitis sites (GP) 9 periodontitis sites (PP) from patients having chronic generalized periodontitis and 5 gingivitis sites from subjects with gingivitis alone (GG).

Patients	Before treatment					After treatment				
	PI	GI	PPD	AL	IL-18	PI	GI	PPD	AL	IL-18
GP										
1	0.7	0.6	2.6	1.0	8.45	0.0	0.5	1.8	1.0	3.72
2	1.1	0.6	1.6	0.6	10.57	0.3	0.0	2.0	1.0	4.46
3	1.0	1.0	2.4	1.2	4.48	0.5	0.6	2.2	1.0	8.09
4	1.6	1.8	2.8	1.4	8.8					
5	1.4	1.2	2.4	0.6	5.21	0.9	1.2	2.2	1.6	9.16
6	0.9	0.9	3.0	0.6	11.28	0.5	1.4	3.0	1.0	9.16
7	1.7	1.9	3.0	0.2	9.51	0.9	1.6	2.4	0.4	13.07
8	1.3	0.9	3.8	3.2	9.51	0.7	0.4	2.8	3.4	8.45
9	1.2	1.3	3.0	1.4	16.17					
PP										
1	1.1	0.8	8.0	9.8	2.26	1.0	0.5	4.4	5.6	12.68
2	0.9	0.7	6.4	6.8	11.28	0.3	0.3	4.4	6.6	8.09
3	1.8	1.4	5.0	7.2	8.8	0.6	0.4	4.2	7.0	7.73
4	1.4	1.5	4.8	5.0	17.9	-	-	-	-	-
5	1.6	1.2	7.6	9.2	21.01	1.0	1.1	6.0	8.8	14.78
6	0.6	0.5	6.6	7.6	28.54	0.9	1.4	5.2	8.0	11.98
7	1.5	1.4	6.8	7.2	12.33	0.9	1.5	6.4	5.8	8.09
8	1.0	0.4	7.8	9.6	24.78	0.5	0.3	6.2	8.7	13.73
9	1.6	1.4	7.2	8.0	10.57	-	-	-	-	-
GG										
1	1.5	1.4	2.3	2.3	21.35	0.3	0.2	2.0	2.5	10.93
2	0.5	1.3	1.6	0.0	5.94	0.7	0.8	1.8	0.0	9.51
3	0.8	0.9	2.4	0.2	11.98	0.5	1.0	1.8	0.4	12.33
4	1.4	1.8	3.0	0.0	25.81	0.8	1.2	1.8	0.0	9.87
5	1.0	1.2	3.0	0.0	9.87	0.3	0.7	2.0	0.2	14.78

0,5 ± 0,2), GP (1,2 ± 0,3 para 0,5 ± 0,3) e PP (1,3 ± 0,4 para 0,7 ± 0,3) 30 AT. Também houve uma redução significante no IG do GG (1,3 ± 0,3 para 0,7 ± 0,4). A profundidade de bolsa reduziu no GG (2,4 ± 0,6 para 1,9 ± 0,1) e no PP (6,7 ± 1,1 para 5,2 ± 0,9) 30 AT. Quando todas as amostras foram analisadas juntas, houve uma redução significante do IL-18 (12,9 ± 7,2 para 10,0 ± 3,1). Esse estudo mostrou que a terapia periodontal não-cirúrgica é eficaz em reduzir os níveis de IL-18 no fluido gengival de sítios inflamados.

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