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Systemic inflammatory markers in young periodontal patients compared with healthy individuals

Markers di infiammazione sistemica in giovani pazienti con parodontite confrontati con controlli sani

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Summary

Aim of this study was to evaluate the pattern of pro-inflammatory cytokines (IL-1 IL-6, IL-8, IL-12, IP-10, IFN-y, TNF-α, MCP-1, MIP-1α, MIP-1β, VEGF) and anti-inflammatory cytokines (IL-4, IL-1RA, IL-10) in young adults (≤ 40 years) with severe periodontitis compared with healthy matched individuals.

The results of this study reported that young periodontal patients showed systemic inflammation with higher levels of Monocyte Chemoattractant Protein-1 (MCP-1), Vascular Endothelial Growth Factor (VEGF) and Interleukin-8 (IL-8) than healthy individuals, thus supporting a systemic effect of periodontal disease.

Riassunto

Il rapporto sistemico fra citochine pro ed anti-infiammatorie in pazienti con parodontite è ancora poco conosciuto. Lo scopo di questo studio era di valutare il livello sistemico di citochine pro-infiammatorie (IL-1β, IL-6, IL-8, IL-12, IP-10, IFN-γ, TNF-α, MCP-1, MIP-1α, MIP-1β, VEGF) ed anti-infiammatorie (IL-4, IL-1RA, IL-10) in giovani adulti (≤ 40 anni) con parodontite grave confrontati con controlli sani.

I risultati di questo studio dimostrano che giovani adulti con parodontite grave presentano livelli sistemici maggiori di Proteina Chemotattica dei Monociti (MCP-1), Interleuchina-8 (IL-8) e Fattore di Crescita Vascolare Endoteliale (VEGF) e supportano un effetto sistemico della parodontite.

Introduction

Periodontal disease is a chronic and multi-factorial infectious disease affecting gingival tissues causing progressive bone loss around teeth (Sanz & Quirynen 2005). Periodontal pathogens and their products elicit an inflammatory response in the gingival tissues, followed by the release of pro-inflammatory proteins from activated immune cells, namely leukocytes, fibroblast or tissue-derived cells (Berglundh & Donati 2005). The inflammatory infiltrate from the gingival tissue can initiate connective tissue/alveolar bone destruction through the activation of several pro-inflammatory cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6), Tumor Necrosis Factor α (TNF-α), interleukin-12 (IL-12) and interferon-γ (IFN-γ). Inflammatory cytokines, rapidly produced by immune cells following microbial exposure, are able to modulate the development of local immune response. Pro-inflammatory cytokines are also involved in the recruitment of inflammatory cells through chemioattractant factors, stimulation of neutrophil degranulation and metalloprotease production, activation of T/B lymphocytes and in the differentiation of osteoclasts (Berglundh & Donati 2005). During microbial infection the binding of cytokines such as IL-1β, IL-6, TNF-α and IFN-γ to cell surface receptors of different cell types enhances the production of such chemotactic cytokines (chemokines) as interleukin-8 (IL-8), monocyte chemoattactant protein-1 (MCP-1), macrophage inflammatory protein 1-α (MIP-1α), macrophage inflammatory protein 1-β (MIP-1β) and interferon inducing protein-10 (IP-10) which attract leukocytes, macrophages and osteoclasts and consequently can provide a positive feedback loop that maintains a sustained inflammatory response. In this compound scenario, regulatory cytokines as interleukin 1 receptor antagonist (IL-1ra), interleukin-4 (IL-4), interleukin-10 (IL-10) may modulate the effects of pro-inflammatory cytokines reducing inflammatory response. The balance between pro-inflammatory and anti-inflammatory mediators may condition connective tissue destruction and progression of periodontitis (Kinane et al. 2003). Toxins and inflammatory mediators from periodontal tissue may reach the bloodstream inducing a possible systemic inflammatory state with higher levels of acute-phase proteins (Loos et al. 2000; D'Aiuto et al. 2004) thus interacting with the etiological pathway of some inflammatory diseases as atherosclerosis.

The relationship between levels of pro-inflammatory/anti-inflammatory cytokines and periodontal disease has been extensively analyzed in crevicular gingival fluid (Silva et al. 2007) but it is poorly investigated at systemic level.

Aim of this study was to evaluate the pattern of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-12, IP-10, IFN-γ, TNF-α, MCP-1, MIP-1α, MIP-1β, VEGF) and anti-inflammatory cytokines (IL-4, IL-1RA, IL-10) in young adults (≤ 40 years) with severe periodontitis compared with healthy individuals.

Material and Methods

Patients

Details of patient population was presented in a companion paper (Cairo et al. 2008). Briefly, patients with age ranging between 18-40 years, body mass index (BMI) ≤27 kg/m^2 and blood-pressure ≤130/80 mm/Hg were enrolled in this study. Patients with systemic disease (e.g, diabetes mellitus or cardiovascular, kidney, liver, or lung disease), a recent history or the presence of other acute or chronic infection, systemic antibiotic treatment within the previous 3 months or any other regular medication, pregnancy and intense physical activity were excluded from the study.

Young patients with severe generalized periodontal disease referred to the Department of Periodontology of Florence University represented the *test group*. Severe periodontitis was defined as at least 30% of sites with clinical loss of attachment and alveolar bone loss exceeding 1/3 of the root in at least 30% of entire dentition.

Control group consisted of healthy individuals without history of periodontal disease (clinical attachment level ≤3mm in each site) referred for oral hygiene procedures. To obtain two homogeneous groups, patients and controls were paired according to gender, age (using a 5-year threshold of tolerance), body mass index (using 1-value as threshold of tolerance), smoking habits (patient and control groups were paired for number of cigarettes/day and exposure to smoking in years). To be considered smokers, patients had to be using tobacco for at least five years. Patients who reported to be smokers for less time were excluded from the study.

All subjects underwent to physical examination. An extensive medical history was determined from each subjects by the interview method. A single periodontist (FC) performed a complete periodontal examination in each patient collecting data on tooth loss, Full Mouth Plaque Score (FMBS), Full Mouth Bleeding Score (FMBS). Probing depth (PD) and clinical attachment level (CAL) were recorded in 6 points/tooth. Each participant signed an informed consent in accordance with the Helsinki Declaration of 1975.

Blood collection and laboratory analysis

Venous blood samples were collected from each patient and control subject by a single venipuncture in the antecubital fossa after an overnight fasting between 8 am and 10 am. Venous blood samples were taken from each subject at least two weeks after periodontal examination and before any periodontal treatment. All laboratory procedures were performed by an experienced operator (AMG), blinded with respect to periodontal conditions.

Whole venous blood was collected in tubes containing ethylenediaminotetracetate (EDTA) 0.17 mol/L and in tubes without anticoagulant. EDTA samples were cen-

trifuged at -4°C and sera samples at room temperature (2500 x g) for 15 minutes. The supernatants were stored in aliquots at –80°C until assays.
IL-1β, IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-12, IP-10, IFN-γ, MCP-1, MIP-1α, MIP-1β, TNF-α, VEGF levels were determined by using the Bio-Plex cytokine assay (Bio-Rad Laboratories Inc, Hercules, CA, USA) according to manufacturer's instructions.

Statistical analysis
Statistical analysis was performed with JMP ® 7.0 SAS Institute Inc. Values are presented as mean ± standard deviation.
Sample size calculation was described in a companion paper (Cairo et al. 2008) and resulted in 45 individuals for each arm. As the variables investigated had a non-gaussian distribution, base-e logarithm-transformed values for cyto-chemokines were used in the analyses, and back transformed for data presentation.
Multiple linear regression analysis was performed using models in which each base-e logarithm-transformed cytochemokine were used as the dependent variable and gender, age, pack-year, BMI, educational level and regular physical activity as potential confounders. Furthermore, the interaction between periodontitis and pack-year was assessed in the models and then eliminated when resulting as not significant.

Results

A total of 90 subjects participated in this study. The test group consisted of 45 systemically healthy individuals affected by severe periodontitis, while the control group comprised 45 systemically healthy matched individuals without clinical signs of periodontal disease. Table 1 showed the clinical characteristics of periodontal patients and control subjects. As expected, significant differences in tooth loss ($p<0.0001$), FMPS ($p<0.0001$) and FMBS ($p<0.0001$) were observed (table 1). Details of descriptive statistics were presented in a companion paper (Cairo et al. 2008).
Among the investigated inflammatory markers, statistically significant higher systemic levels of IL-8 ($p=0.0488$), MCP-1 ($p=0.0062$) and VEGF ($p=0.0056$) were detected in periodontal patients than in controls (table 2). The interaction between periodontitis and smoking habits was not significant.

Discussion

In the last decade a growing body of evidence has suggested that periodontal disease is not only a localized infection leading to loss of connective tissue attachment/bone around teeth but it may be also able to trigger a systemic inflammation.

Some studies showed that patients with severe periodontitis have increased levels of C-reactive protein, IL-1, IL-6 and hyperfibrinogenaemia when compared with healthy controls (Loos et al. 2000, D'Aiuto et al. 2004, Cairo et al. 2008), even if these markers are not specifically related with periodontal disease. These evidences support the hypothesis that systemic inflammation due to severe periodontitis may influence the pathogenesis of atherosclerosis (for review see Paquette et al. 2007).

Using extensive array of cytokines, the present case-controlled study explored the inflammatory profile of young adults (≤40years) with severe periodontitis. The results of the study showed that young periodontal patients had an exuberant pro-inflammatory response not sufficiently counteracted by the anti-inflammatory cytokines. Higher levels of IL-8, MCP-1 and VEGF were detected in periodontal patients than in controls.

These findings corroborate the results of previous studies reporting Interleukin-8 (IL-8) and Monocyte Chemoattractant Protein-1 **(**MCP-1) in gingival crevicular fluid from periodontal lesions (Silva et al. 2007), thus supporting a systemic effect of periodontal disease. These cytokines are involved in an amplification loop leading to chemotaxis and differentiation of osteoclast precursors into osteoclasts and then stimulating bone resorption. Furthermore, several papers support the pivotal role of cytokines and chemokines in the pathogenesis of atherosclerosis. The endothelial expression of MCP-1 seem to be crucial especially in the earliest cellular responses of atherogenesis. In fact, many atherogenic stimuli can exert their effects inducing MCP-1 expression within the vascular wall, leading to the recruitment of monocytes into atherosclerotic lesions and in the formation of intimal hyperplasia after arterial injury. In addition, IL-8 is able to attract and activate neutrophils in the inflammatory site (for review see Charo & Taubman 2004).

Vascular endothelial growth factor (VEGF) is a key regulator of physiologic angiogenesis during embryogenesis. VEGF is also involved in endothelial cell growth and vascular permeability. Experimental studies supported its role in pathogenesis of periodontal disease leading to alveolar bone loss via activation of cyclooxygenase (COX)-2 system (Oliveira et al. 2008). Deregulated VEGF expression may contributed to the pathogenesis of several diseases characterized by abnormal angiogenesis (atherosclerosis, cancer, diabetic retinopathy etc). VEGF expressed from endothelial cells and macrophage-lineage cells binds and activates two tyrosine kinase receptors, VEGFR1 (Flt-1) and VEGFR2 (KDR/Flk-1), leading to physiological as well as pathological angiogenesis and inflammation.
In conclusion, this study provides new insights on systemic inflammation in periodontal patients showing that cytokines such as MCP-1, VEGF and IL-8 are inflammatory markers in young adults with severe periodontitis.

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Table 1. Clinical characteristics of periodontal patients and control subjects (t-test and Fisher exact test)

Variables	Controls N= 45	Periodontal patients N=45	p-value
Age (yrs)	33.8 ± 3.3	36.3 ± 3.6	*0.0007**
Gender (females)	21 (47%)	21 (47%)	--
Number cigarettes/die	5.3 ± 6.9	5.3 ± 6.9	--
Smoking habits >5years (yrs)	18 (40%)	18 (40%)	--
Pack/year	3.7 ± 4.9	4.1 ± 5.6	*0.6682**
Mean CAL (mm)	2.2 ± 0.1	4.7 ± 0.7	--
Mean Probing Depth (mm)	2.2 ± 0.1	4.3 ± 0.7	--
Tooth Loss	0.3 ± 0.5	2.3 ± 2.3	*<0.0001**
FMPS (%)	23.0 ± 9.6	50.0 ±20.4	*<0.0001**
FMBS (%)	18.0 ± 8.6	47.0 ± 16.4	*<0.0001**
Number of sites with CAL≥ 4mm	0.4 ± 0.6	96.9 ± 27.5	--
Number of sites with PD≥ 4mm	0.0 ± 0.0	72.6 ± 23.8	--
Family history of cardiovascular disease	4 (9%)	19 (42%)	*0.0005#*
Regular physical activity	18 (40%)	15 (33%)	*0.6621#*
BMI (kg/m^2)	22.5 ± 2.1	22.4 ± 2.0	*0.9551**
Educational level (higher than high school)	28 (62%)	8 (18%)	*<0.0001#*

Data are expressed as mean and standard deviation or number and percentage n, (%)
** t-test*
 # Fisher's exact test

Table 2. Cyto-chemokine levels in periodontal patients and controls (multiple linear regression analysis)

Variables	Controls (n=45)	Periodontal patients (n=45)	p-value
IL-1β (pg/mL)	25.8± 8.3	48.5± 78.1	*0.4019*
IL-6 (pg/mL)	72.9 ± 73.2	2209.8 ± 10292.2	*0.1391*
IL-12 (pg/mL)	92.7± 108.9	250.8± 884.4	*0.8959*
TNF-α (pg/mL)	469.3± 85.4	650.8± 1028.5	*0.8235*
IFN-γ (pg/mL)	609.3± 256.5	2009.9± 9504.9	*0.6952*
IL-1ra (pg/mL)	945.2± 834.0	3912.7± 15185.5	*0.0976*
IL-4 (pg/mL)	57.2 ± 20.5	112.6± 324.5	*0.9422*
IL-10 (pg/mL)	32.3± 42.7	112.7± 479.4	*0.7090*
IP-10 (pg/mL)	4578.5± 3394.7	3691.6± 1996.2	*0.0871*
MCP-1 (pg/mL)	754.8± 516.1	1347.3± 1290.0	*0.0062*
MIP-1 α (pg/mL)	55.8± 23.4	117.4± 258.7	*0.1159*
MIP-1 β (pg/mL)	931.5± 501.0	1701.3± 2886.3	*0.0812*
IL-8 (pg/mL)	860.9±4783.9	1059.5±2395.9	*0.0488*
VEGF (pg/mL)	613.7± 643.3	1249.3± 1068.2	*0.0056*

Data are expressed as mean and standard deviation

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