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Original Article

Systemic expression of genes related to inflammation and lipid metabolism in patients with dyslipidemia, type 2 diabetes mellitus and chronic periodontitis

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ABSTRACT

Inflammatory diseases, as periodontal disease (PD), has been associated with disturbance of lipid and glycemic metabolisms, as demonstrated by the increasing of PD patients with type 2 diabetes mellitus (T2D) and/or dyslipidemia comorbidities. We aimed to investigate the expression of inflammation and lipid metabolism genes, and correlations among clinical and biochemical characteristics in normoglycemic or T2D patients with dyslipidemia and PD, in comparison with healthy individuals. Five groups of 30 individuals each (150 patients) were formed based upon T2D, dyslipidemic and periodontal status. Blood analyses of lipid and glycemic profiles were carried out, and the gene expression was assessed by RT-qPCR. The systemic expression of *IL6, TNFA* and *LEP* genes were significantly higher in T2D, dyslipidemia and PD patients, while the *PECAM1* gene showed the opposite. Higher *RETN* levels were found in patients with T2D independently of their glycemic control status. There were positive correlations between: *TNFA, LEP* and *RETN* with worse periodontal parameters; *IL6, TNFA, ADIPOR1, LEP and RETN* with waist-to-hip ratio; glycemic parameters with *RETN;* total cholesterol and triglycerides with *LEP* expression. We conclude that pro-inflammatory cytokines were related with worse lipid, glycemic and periodontal parameters, reinforcing that a hyper-inflammatory status connects systemic and oral inflammatory diseases.

1. Introduction

Patients with diabetes have much higher morbidity and mortality than the general population because of the complications associated with the disease. Diabetes is a well-established independent risk factor for cardiovascular diseases (CVD), since atherosclerotic cardiovascular disease is the most common macrovascular complication in diabetic patients [1]. The higher incidence of CVD in diabetic patients may be related to endothelial damage caused by hyperglycemia, traditional risk factors (smoking, dyslipidemia, and hypertension), or disturbed metabolism (oxidative stress, decreased nitric oxide production, and chronic inflammation) [1].

Dyslipidemia, which is characterized by a spectrum of metabolic quantitative and qualitative changes in lipids and lipoproteins [2], has been highly investigated in patients with diabetes mellitus [3]. In the Framingham Heart Study, the prevalence of high plasma triglyceride levels in individuals with diabetes mellitus was significantly higher than in those without diabetes mellitus [4]. The prevalence of low HDL-C cholesterol level in those with diabetes mellitus was almost twice as high as the prevalence in nondiabetic individuals [4].

The underlying pathophysiology of diabetic dyslipidemia is

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complex and still not well understood; nevertheless, a large body of evidence suggests that insulin resistance has a central role in the development of this condition [5,6]. Currently, the relationship between lipid and glycemic levels with inflammation has become a focus of research in several studies. Meta-analysis studies observed that inflammatory diseases, including periodontal disease (PD), are associated with disturbance of the lipid and glycemic metabolism of patients [7,8].

Since 1999 the relationship between PD and dyslipidemia has been investigated, considering the hypothesis that periodontitis could alter serum lipid levels [9]. Today, chronic periodontitis (CP) has been identified as the sixth complication of diabetes alongside retinopathy, nephropathy, neuropathy, macrovascular disease and poor wound healing [10]. A recent meta-analysis including 19 studies suggested that PD is significantly associated with reduction of HDL and elevation of LDL and triglyceride concentrations [7]. The biological rationale for this association is that PD, as a chronic infectious disease, as well as T2D, produces high levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-alpha) and interleukin 6 (IL-6), which in turn, lead to alteration of the serum lipid parameters, thereby promoting hyperlipidemia [11]. For the other way, when serum lipid levels are elevated toward the upper limit of the normal physiologic range, it alters the immune cell function and therefore increase the production of pro-inflammatory cytokines [12]. The release of pro-inflammatory cytokines and interleukin is believed to compromise tissue response and affect wound healing, thereby increasing the susceptibility to periodontitis [13]. Soory (2012) had revised the interrelationship among diabetes mellitus, periodontal diseases, obesity and cardiometabolic disorders, showing that patients affected by at least one of these pathological conditions present a hyper-inflammatory state, and the common risk markers lead to a bidirectional relationship among them [14].

In spite of the near relationship among pro-inflammatory cytokines associated with PD and T2D, such as TNF-α and IL-6 that regulate the adipokines leptin (*LEP*), adiponectin (*ADIPOQ*) and resistin (*RETN*) [15], all related with lipid metabolism, insulin sensitivity and inflammation [16], there are no studies investigating the expression of these genes in subjects concomitantly affected by T2D, dyslipidemia and PD. In addition, other genes such as platelet and endothelial cell adhesion molecule 1 (*PECAM1*) and CCAAT/Enhancer binding protein delta (*CEBPD*) also regulate the TNF-α, IL-6 and other inflammatory cytokines [17–19]. In view of the lack of literature, and because it has been increasingly common to find diabetic dyslipidemic patients that are also affected by CP, our hypothesis is that lipid profile, glycemic control and periodontal status can alter the systemic expression of these genes associates with lipid metabolism.

Therefore, the aim of the present study was to evaluate the expression of the *IL6*, *TNFA*, *LEP*, *ADIPOQ*, *ADIPOR1*, *RETN*, *CEBPD* and *PECAM1* important genes in the context of inflammation and lipid metabolism, in poorly or well-controlled T2D patients and in normoglycemic individuals, both conditions associated with dyslipidemia and chronic periodontitis. Importantly, correlation analyses were made in order to assess the mutual expression influence of these genes to better understand their biological interrelationship.

2. Material E methods

2.1. Study population

The study was approved by the Ethics in Human Research Committee of the Araraquara School of Dentistry (UNESP - São Paulo State University, Araraquara, Brazil; Protocol number 50/06) and was conducted according to the ethical principles of the Declaration of Helsinki during three years (2009 and 2011). All volunteers were informed about the aims and methods of this study, and they provided their written consent to participate.

We evaluated 1788 patients, age ranging from 35 to 60 years, similar socioeconomic level and with at least 15 natural teeth. Because the patients enrolled here were the same as those investigated in previous studies of our research group, detailed information regarding recruiting, clinical inclusion criteria, power analysis, physical and periodontal examination, and biochemical metabolic evaluations can be found in Corbi et al. (2014) [20] and Nepomuceno et al. (2017) [21]. The diagnosis criteria for dyslipidemia, T2D and PD are described in Fig. 1.

From the total of 1788 patients screened, 150 of them obeyed the research inclusion criteria described in Fig. 1, and were divided into five groups containing 30 individuals each, based upon T2D, dyslipidemic and periodontal status: (Group 1) poorly controlled T2D and dyslipidemic patients with chronic periodontal disease; (Group 2) well-controlled T2D and dyslipidemic patients with chronic periodontal disease; (Group 3) normoglycemic individuals with dyslipidemia and chronic periodontal disease; (Group 4) systemically healthy individuals with chronic periodontal disease; and (G5) systemically healthy individuals without chronic periodontal disease.

2.2. Reverse transcriptase-quantitative PCR (RT-qPCR)

Peripheral venous blood collected from each subject was utilized to extract total RNA, and the cDNA was synthesized as described in Nepomuceno et al. (2017) [21]. All reactions were performed in duplicate utilizing TaqMan® gene expression assays in the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The expression of the target genes *IL6*, *TNFA*, *ADIPOQ*, *ADIPOR1*, *LEP*, *RETN*, *CEBPD* and *PECAM1* was investigated after normalization to the *GAPDH* endogenous control gene, using the comparative Cτ ($2^{-\Delta C\tau}$) method.

2.3. Statistical analysis

The distribution of the data was evaluated by D'Agostino–Pearson omnibus normality test. Comparisons between all groups for non-normally distributed variables (e.g. $2^{-\Delta C\tau}$ values of gene expression) were performed by the Kruskal–Wallis test, and Dunn test was used to correct for multiple comparisons. For normally distributed variables, we performed one-way analysis of variance (ANOVA) test with Holm–Sidak multiple comparison test. The general characteristics of each group are described with the mean and standard deviation (SD). The significance level was set at $\alpha = 0.05$. GraphPad Prism 5.0 software (GraphPad Inc., USA) was used to perform these statistical analyses.

To investigate correlations among the genes and between gene expression and various studied parameters we performed the Spearman's rank correlation test adjusted for age and gender. To identify potential confounders, we performed multiple linear regression analyses adjusted for age and gender. This was done for each parameter (fasting glucose, HbA1c, HOMA IR, total cholesterol, HDL, LDL, triglycerides, bleeding on probing, percentage of sites with probing depth ≥ 6 mm and percentage of sites with attachment loss ≥ 5 mm) treating genes expression, age and BMI as continuous variables and gender as categorical variables. The correlation and regression analyses were performed using SPSS® IBM software version 24, and the significance level was set at $\alpha = 0.05$.

3. Results

Because this present study aimed to assess correlations among

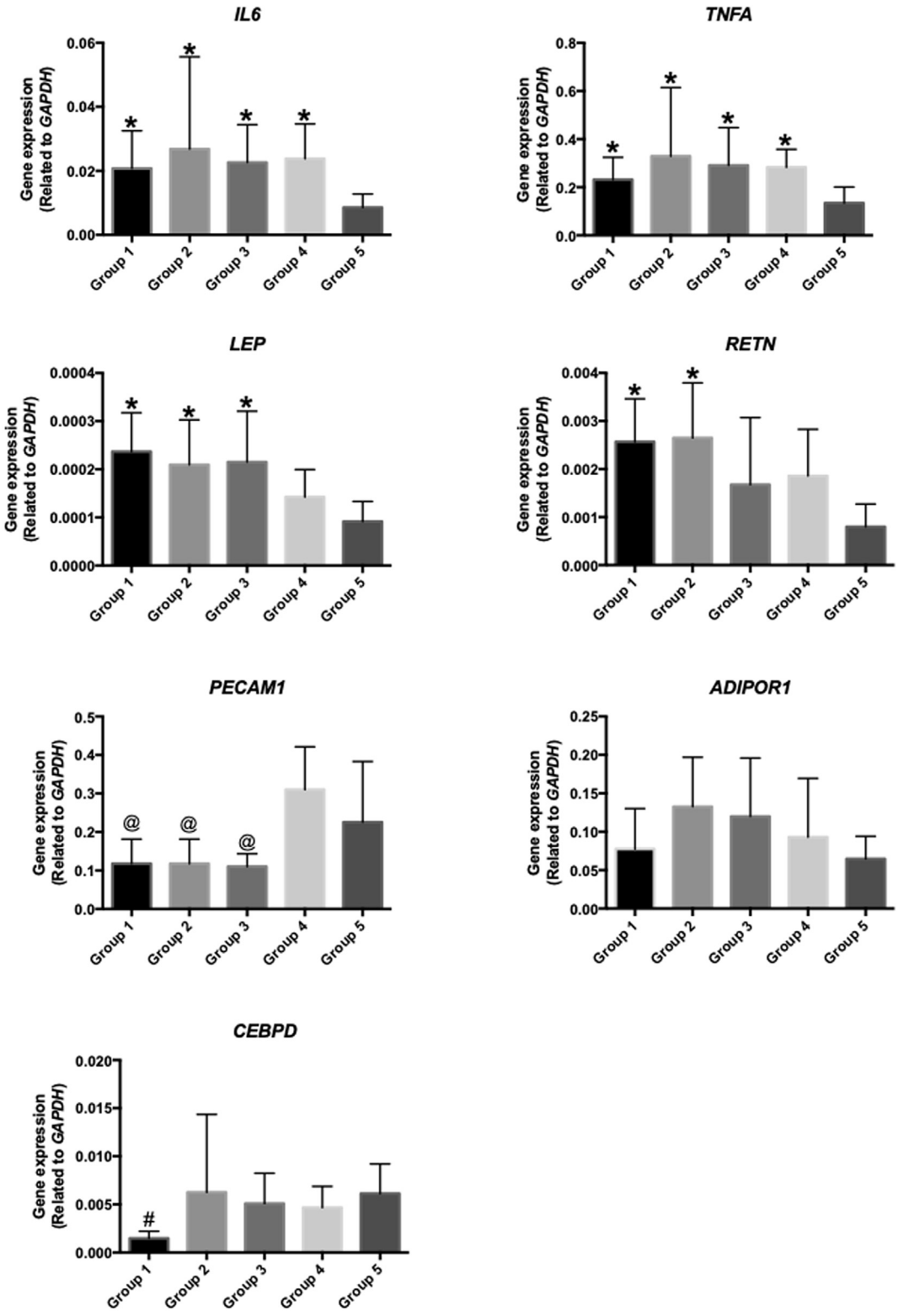

Fig. 1. Criteria for the diagnosis of diabetes mellitus type 2,dyslipidemia chronic periodontal disease.

expression in relevant metabolic genes and clinical and biochemical characteristics, the patients enrolled here were the same as presented in Corbi et al. (2014) [20] and Nepomuceno et al. (2017) [21]. Therefore, the patients' demographic, physical, biochemical and periodontal characteristics can be found in Ref. [21]. Fasting glucose and HbA1c were significantly higher in Group 1, confirming the poor glycemic control; which were higher in Group 2 group in comparison with the nondiabetics groups (Groups 3, 4 and 5). Insulin levels showed no significant differences between Groups 1 and 2. As expected, the total cholesterol, LDL-cholesterol and

triglycerides levels were higher in Groups 1, 2 and 3 than Groups 4 and 5, confirming the clinical dyslipidemia. The periodontal tissue destruction (including bone loss) and local inflammation were significantly more severe in T2D patients, particularly in Group 1, which presented high percentage of periodontal sites with bleeding on probing, probing depth ≥ 6 mm, clinical attachment loss ≥ 5 mm and suppuration. The groups with dyslipidemia (Groups 1, 2 and 3) showed significant difference in relation to the presence of deeper periodontal sites when compared to groups without dyslipidemia (Groups 4 and 5).

Regarding gene expression analysis (Fig. 2), *IL6* and *TNFA* genes were significantly more expressed in patients with PD (Groups 1, 2, 3 and 4). We observed statistically significant higher mRNA levels of *LEP* in patients with dyslipidemia (Groups 1, 2 and 3) and higher *RETN* in patients with T2D (Groups 1 and 2) independently of their glycemic control status (Fig. 2). Opposite to the *LEP* gene, the *PECAM1* gene was significantly lower expressed in dyslipidemic groups (Groups 1, 2 and 3), also independently of the glycemic status. Considering the gene expression of adipokines, *ADIPOQ* was not expressed on circulating PBMC, and its receptor, the *ADIPOR1*, had the mRNA levels similar among the groups. Moreover, the mRNA levels of *CEBPD* gene was statistically significant lower in the Group 1 in comparison with groups 2, 3, 4 and 5 (Fig. 2).

We developed correlation analyses between the gene's expression (Table 1). The *IL6* and *TNFA* genes correlated positively with each other (r = 0.64) and with *LEP* and mainly *CEBPD* (r = 0.63 and 0.41 respectively). In addition, the expression of *CEBPD* was negatively correlated with *RETN*. Furthermore, *LEP* negatively correlated with *PECAM1*.

Gene expression was significantly correlated with physical parameters and with glycemic and lipid profiles (Table 2). Waist-to-hip ratio was positively correlated with *IL6*, *TNFA*, *ADIPOR1*, *LEP* and mainly *RETN* (r = 0.81), and negatively correlated with *PECAM1*. Glycemic parameters (fasting glucose levels and HbA1c) were positively correlated with *RETN* and negatively with *CEBPD* and *PECAM1*. Total cholesterol and triglycerides were positively correlated with *LEP* expression, nevertheless, total cholesterol, LDL and triglycerides were negatively correlated with *PECAM1*.

Spearman's correlation coefficients are shown (r; α = 5%) adjusted for age and gender. *Significant correlations ($p < 0.05$). **Significant correlations ($p < 0.001$). BMI: Body Mass Index; HbA1c: Glycated Haemoglobin; HOMA: Homeostasis Model Assessment of Insulin Resistance.BP: bleeding on probing; PPD: periodontal pocket depth; CAL: clinical attachment loss.

Significant correlations were also observed between gene expression and periodontal parameters (Table 3). Increases in the percentage of the periodontal clinical parameters (visible plaque, marginal bleeding, BP, PPD ≥ 6 mm, NI ≥ 5 mm, and suppuration) indicate the severity of chronic periodontitis [22]. Their main findings were the positive correlation between *TNFA* and adipokines (*LEP* and *RETN*) with the visible plaque, marginal bleeding, BP, suppuration, PPD ≥ 6 mm and mainly CAL ≥ 5 mm (r = 0.53 for *LEP*); besides negative correlation with PPD ≤ 3 mm and CAL ≤ 2 mm. Nevertheless, the *CEBPD* and *PECAM1* expressions were negatively correlated with periodontal clinical parameters that indicate the severity of chronic periodontitis.

Table 1
Adjusted correlations of expression between the investigated genes.

	IL6	*TNFA*	*ADIPOR1*	*LEP*	*RETN*	*CEBPD*	*PECAM1*
IL6	1.00						
TNFA	0.64**	1.00					
ADIPOR1	0.17	0.05	1.00				
LEP	0.42**	0.41**	−0.13	1.00			
RETN	0.12	−0.01	0.14	0.29	1.00		
CEBPD	0.63**	0.41**	0.18	−0.08	−0.40**	1.00	
PECAM1	0.09	0.16	0.14	−0.30*	−0.06	0.08	1.00

Spearman's correlation coefficients are shown (r; α = 5%) adjusted for age and gender. *Significant correlations ($p < 0.05$). **Significant correlations ($p < 0.001$).

We performed multiple linear regression analyses adjusted for age and gender and the results confirmed the overall association between glycemic, lipid and periodontal parameters with expression of some the investigated genes (model a, Table 3). We found a significantly increased *IL6* expression associated with increasing HbA1c and HOMA IR (β = 0.55 and β = 0.74, respectively); and the reduced *CEBPD* expression with increasing fasting glucose levels, HbA1c and HOMA IR (β = −0.77, β = −0.80, β = −0.51, respectively).

LEP mRNA expression was the only gene significantly affected by increasing lipids parameters. The increase of triglycerides upregulates the *LEP* expression (β = 0.70). The expression of *IL6* was significantly upregulated with increasing the percentage of PPD ≥ 6 mm and CAL ≥ 5 mm (β = 0.79 and β = 0.51, respectively), whereas significant downregulation of the *ADIPOR1* and *CEBPD* genes were associated with increasing PPD ≥ 6 mm (%) (β = −0.44 for both genes).

Additional multiple linear regression analyses were performed including BMI besides age and gender in the adjustment to confirm the overall association between the periodontal and metabolic parameters with genes expressions (model b, Table 3). Observing this additional adjusted analysis, the statistical results remained significant, excepting regarding the associations of the *ADIPOR1* with PPD ≥ 6 mm, and the *CEBPD* with HOMA IR.

Type 2 Diabetes Mellitus	Non-diabetics (normoglycemic)	fasting glucose levels <100 mg/dL and HbA1c <6.5%.
	Diabetic - well-controlled	fasting glucose levels ≥ 100 mg/dL and HbA1c ≥ 8.5%
	Diabetic - poorly controlled	fasting glucose levels ≥ 100 mg/dL and HbAc1 < 7.0%
	According to ADA - American Diabetes Association, 2012	
Dyslipidemia	According to the National Cholesterol Educational Program Adult Treatment III (ATP III), 2001.	Abnormal levels of at least one of the parameters: TC ≥ 240 mg/dL, LDL ≥ 160 mg/dL, HDL ≤ 40 mg/dL, and TG ≥ 200 mg/dL
Chronic Periodontal Disease	(Defined by the American Academy of Periodontology, 1999)	At least four nonadjacent teeth with PPD ≥ 6mm; CAL ≥ 4mm and BP.

HbA1c: Glycated Haemoglobin; TC: Total Cholesterol; TG: Triglycerides; PPD: periodontal pocket depth; CAL: clinical attachment loss; BP: bleeding on probing.

Fig. 2. Expression analysis of the *1L6,TNFA,LEP,RETN, ADIPOR1,CEBPD* and *PECAM1* genes in groups1,2,3,4 and 5.
Data represent the mean ±
SD of $2^{-\Delta C\tau}$ value of gene expression All value were normalized to GAPDH.*$p \leq 0.05$ compared to Group 5; @$p \leq 0.05$ compared to group 4 and 5;$^{\#}p \leq 0.05$ compared to Group 2,3,4,5; Comparisons between all groups were performed by the kruskal-Wallis test, and Dunn test was used to correct for multiple comparisons.

Table 2
Adjusted correlations between gene expression and physical parameters, glycemic and lipid profiles, as well between gene expression and periodontal parameters.

	Physical parameters			Glycemic and lipid profiles					
	BMI (m/kg2)	Waist-to-hip ratio (cm)	Waist circumfe-rence (cm)	Fasting glucose (mg/dl)	HbA1c (%)	HOMA IR	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	Triglycerides (mg/dl)
IL6	0.05	0.29**	0.03	−0.02	0.03	−0.02	−0.04	0.04	−0.07	0.00
TNFA	0.00	0.31**	−0.05	−0.03	−0.02	−0.09	0.00	−0.03	−0.03	0.06
ADIPOR1	0.11	0.23*	0.10	−0.11	−0.15	−0.02	−0.05	−0.10	−0.07	0.07
LEP	0.02	0.26*	−0.10	0.20	0.24	0.08	0.26*	0.12	0.11	0.27*
RETN	0.20	0.81**	0.22	0.33**	0.33**	0.04	−0.02	−0.21	−0.08	0.16
CEBPD	−0.11	−0.05	−0.02	−0.32**	−0.32**	−0.19	−0.07	0.27*	0.00	−0.31**
PECAM1	−0.28**	−0.32**	−0.20*	−0.26**	−0.27**	−0.05	−0.39**	−0.03	−0.28**	−0.30**
	Visible Plaque (% sites)	**Marginal Bleeding** (% sites)	**BP** (% sites)	**PPD ≤ 3 mm** (% sites)	**PPD 4-5 mm** (% sites)	**PPD ≥ 6 mm** (% sites)	**CAL ≤ 2 mm** (% sites)	**CAL 3-4 mm** (% sites)	**CAL ≥ 5 mm** (% sites)	**Suppuration** (nº sites)
IL6	0.14	0.23*	0.16	−0.23*	0.18	0.13	−0.23*	0.07	0.20	0.10
TNFA	0.17	0.19	0.21	−0.23*	0.28*	0.00	−0.34**	0.16	0.27*	0.06
ADIPOR1	0.08	0.03	0.03	−0.02	0.08	−0.07	−0.07	0.12	−0.02	−0.07
LEP	0.46**	0.35**	0.40**	−0.45**	0.18	0.43**	−0.36**	−0.20	0.53**	0.34**
RETN	0.25*	0.42**	0.30**	−0.34**	0.27*	0.18	−0.33**	0.01	0.33**	0.31**
CEBPD	−0.16	−0.22*	−0.21	0.21*	−0.11	−0.23*	0.14	0.03	−0.17	−0.23*
PECAM1	−0.22*	−0.25**	−0.25**	0.15	−0.03	−0.22*	0.05	0.20*	−0.20*	−0.08

4. Discussion

The evidence regarding the association between diabetes and dyslipidemia with periodontal disease is increasing and has been shown in numerous studies [7,23,24]. The findings of the present study indicated that diabetes resulted in increased mRNA levels of *RETN*; dyslipidemia resulted in increased *LEP* and reduced *PECAM1* mRNA levels; and periodontal disease in increased *IL6* and *TNFA* mRNA levels. Furthermore, data analysis suggests that elevated levels of pro-inflammatory cytokines, such as *IL6*, *TNFA*, *LEP* and *RETN*, and low levels of *CEBPD* and *PECAM1* are correlated with sites showing periodontal destruction.

It has been shown through gene expression studies in humans that resistin is predominantly expressed in PBMCs, macrophages, and bone marrow [25]. Plasma resistin levels were found to be significantly increased in diabetic patients as compared to non-diabetic patients, independently of glycemic control [26]. The same result we found to mRNA levels in this study. No significant difference in the resistin levels between patients with and without T2D was demonstrated; however the resistin levels are increased in patients with overweight T2D compared to those with non-overweight [27]. These results are complementary to ours here showed, once the *RETN* was positively correlated with waist-to-hip ratio and glycemic parameters (fasting glucose levels and HbA1c), indicating that this gene is positively correlated with important parameters for T2D and obesity.

In addition to the relationship of resistin with diabetes and obesity, in our study there was positive correlation between *RETN* with the visible plaque, marginal bleeding, BP, suppuration, and CAL $\geq$5 mm. Our correlation analyses result contribute to currently available literature that suggests that the levels of resistin are increased in the patients with chronic periodontitis compared to the clinically healthy controls [28]. Resistin induces insulin resistance. Increased resistin levels in periodontitis may thus be considered to pose a risk for diabetes by decreasing the insulin sensitivity. Thus, periodontitis might lead to development of type II diabetes or diabetes might influence the occurrence or progression of periodontitis [29]. Obesity, which is another important risk factor for diabetes type 2, has also been linked positively to periodontitis [29]. Resistin may play a more important role in inflammatory processes rather than in processes involved in adiposity and glucose homeostasis [30], and may serve as one of the potential biomarkers for periodontitis with other systemic diseases such as diabetes and obesity [28].

Leptin is an amino acid protein synthesized and secreted mainly by adipocytes, but it is also expressed in PBMCs [31]. Serum leptin concentrations reflect the amount of energy reserves stored in adipose tissue. Available literature provides evidence that leptin resistance (higher serum level of leptin) is a cause of developing hypercholesterolemia, atherosclerosis and related cardiovascular disease, and it is significantly associated with abdominal obesity and hypertriglyceridemia [32]. Some studies revealed a relationship between leptin and the parameters of a lipid profile [31]. In addition, leptin plays an important role in the regulation of feeding behavior and is closely associated and positively correlated with body mass index (BMI) [33]. In the present study, there was a statistically significant higher mRNA level of *LEP* in patients with dyslipidemia (Groups 1, 2 and 3). Besides that, we found a positive correlation of leptin with total cholesterol and triglycerides; and the increase of triglycerides upregulates the *LEP* expression, after multiple linear regression analysis. Although we did not find a positive correlation with BMI, there was found a positive correlation between leptin and waist-to-hip ratio.

This study found a positive correlation between *LEP* with the visible plaque, marginal bleeding, BP, suppuration, PPD $\geq$ 6 mm and

Table 3
Multiple linear regression analyses with glycemic, lipid and periodontal parameters as covariants.

Model a. Multiple linear regression adjusted for gender and age									
	IL6	*TNFA*	*ADIPOR1*	*LEP*	*RETN*		*CEBPD*		*PECAM1*
Fasting glucose (mg/dl)	0.45	0.29	−0.33	0.02	−0.03		−0.77*		0.12
HbA1c (%)	0.55*	0.35	−0.22	−0.03	−0.24		−0.80*		0.20
HOMA IR	0.74*	0.23	−0.11	−0.43	−0.18		−0.51*		0.02
Total cholesterol (mg/dl)	0.20	0.03	−0.04	−0.06		−0.13	0.17	−0.25	
HDL cholesterol (mg/dl)	0.04	0.05	−0.25	−0.17	0.02		0.35		−0.18
LDL cholesterol (mg/dl)	0.32	0.05	−0.06	−0.32	−0.13		0.21		−0.11
Triglycerides (mg/dl)	−0.39	0.12	0.20	0.70*	−0.13		−0.34		−0.24
PPD ≥ 6 mm (% sites)	0.79*	−0.01	−0.44*	0.17	0.12		−0.44*		0.22
CAL ≥ 5 mm (% sites)	0.51*	0.29	−0.19	0.30	−0.04		−0.36		0.16
Model b. Multiple linear regression adjusted for BMI, gender and age									
Fasting glucose (mg/dl)	0.48	0.25	−0.36	0.00	−0.04		−0.74*		0.14
HbA1c (%)	0.60*	0.27	−0.27	−0.07	−0.27		−0.76*		0.24
HOMA IR	0.82*	0.11	−0.20	−0.51	−0.21		−0.44		0.11
Total cholesterol (mg/dl)	0.16	0.08	−0.01	−0.03	−0.11		0.14		−0.28
HDL cholesterol (mg/dl)	−0.07	0.21	−0.15	−0.09	0.07		0.25		−0.26
LDL cholesterol (mg/dl)	0.26	0.14	0.00	−0.27	−0.10		0.16		−0.16
Triglycerides (mg/dl)	−0.27	−0.06	0.09	0.61*	−0.19		−0.23		−0.15
PPD ≥ 6 mm (% sites)	0.74*	0.08	−0.38	0.21	0.14		−0.49*		0.17
CAL ≥ 5 mm (% sites)	0.51*	0.28	−0.20	0.30	−0.04		−0.36		0.17

Partial standardized regression coefficient (b; 95%CI) adjusted for age and gender (model a) or age, gender and BMI (model b). *Significant correlations ($p < 0.05$). BMI: Body Mass Index; HbA1c: Glycated Haemoglobin; HOMA IR: Homeostasis Model Assessement of Insulin Resistence; PPD: periodontal pocket depth; CAL: clinical attachment loss.

mainly CAL ≥5 mm. Leptin is considered to be a proinflammatory cytokine involved in the inflammatory response as it modulates the function of immunocytes such as T-cells, monocytes, and natural killer cells [34], important cells in immune response in periodontal tissues. PD was showed as an independent contributor to high levels of serum leptin [34]. Leptin concentration in serum increases progressively from health to periodontitis; therefore, subjects who suffer from chronic periodontitis have higher serum leptin levels compared to periodontally healthy individuals [35]. Besides that, previous studies have reported an increased leptin circulation during inflammation periods, which was modulated by lipopolysaccharide (LPS) and cytokines such as TNF-α and IL-1 [36]. In addition, it was reported that leptin increased the IL-6 production [37]. In agreement, the *IL6* and *TNFA* genes mRNA levels were positively correlated with *LEP* in our study.

Opposite to *LEP*, the *PECAM1* gene was significantly lower expressed in dyslipidemic patients investigated here; as well the *LEP* mRNA levels were negatively correlated with *PECAM1*. PECAM-1 is a cell adhesion and signaling receptor of the Ig-superfamily that is a major constituent of the endothelial cell intercellular junction and plays a role in neutrophil recruitment at inflammatory sites [38]. There is good evidence suggesting that PECAM-1 is a key participant in the adhesion cascade leading to extravasation of leukocytes during the inflammatory process [38] and related with events leading to atherosclerosis [39]. PECAM-1 is also expressed in most cells of the hematopoietic lineage including monocytes/macrophages, neutrophils, and certain lymphocyte subsets [40]. A prominent function of PECAM-1 is to dampen inflammatory responses, one mechanism being by lowering levels of pro-inflammatory cytokines [41]. This is in agreement with our findings of higher expression of pro-inflammatory markers in all the groups with T2D and dyslipidemia (Groups 1 and 2) and Group 3 with dyslipidemia, while the *PECAM1* showed lower expression in the same groups. To our knowledge, this is the first study to demonstrate the association of lower *PECAM1* gene expression in patients with dyslipidemia.

Local infection is considered to be a contributor to the systemic inflammation burden [42]. IL-6 and TNF-α cytokines are implicated in the destructive process of periodontitis [43] and are released locally and systemically from macrophages [44]. Some studies have demonstrated that serum TNF-α and IL-6 levels are elevated in severe chronic periodontitis subjects, including in PBMC cells [42,45]. Interestingly in the present study, *IL6* and *TNFA* genes were significantly more expressed by PBMCs of patients with chronic periodontitis. Pearson's correlation analysis showed that both genes are positively correlated to each other. Additionally, after multiple logistic regression adjusted for BMI, gender and age, we found a significantly increased *IL6* expression associated with increasing HbA1c and the percentage of PPD ≥ 6 mm and CAL ≥ 5 mm. TNF-α is the important cytokine that is secreted by leukocytes, macrophages and lymphocytes in the local lesion of periodontitis [42]. The possible mechanisms that connect focal infection in periodontitis with systemic cytokine levels might be that the TNF-α stimulated the expression of IL-6 [45,46], showing the relationship between both cytokines and the importance to PD. It is believed that the *IL6* expression in systemic circulation is higher in patients with inflamed periodontal sites due to microbial stimuli or a host response [47]. This mediator interferes with the function of insulin receptors and thereby derange the process of insulin signaling [48], which may justify the increased IL6 expression associated with increasing HbA1c in this present study.

The *IL6* and *TNFA* genes correlated positively not only with each other, but also with the transcription factor CCAAT/enhancer binding protein delta (*CEBPD*; also known as C/EBPdelta, CRP3, CELF, and NF-IL6β). *CEBPD* is known to regulate or coregulate a wide range of inflammatory mediators and participate in signaling by IL-1α, IL-6 and TNF-α [49]. To our knowledge, this is the first study to report the association of this gene with PD. The *CEBPD* expression was negatively correlated with periodontal clinical parameters that indicate the severity of chronic periodontitis, as demonstrated by the significant downregulation of the *CEBPD* associated with increasing PPD ≥ 6 mm (%) through multiple linear regression analyses adjusted for BMI, age and gender. Although the studies suggested that *CEBPD* contributes to proinflammation, the physiological function of *CEBPD* and its downstream targets in inflammation diseases are poorly understood [50].

5. Conclusions

We concluded that *RETN* was higher expressed in patients with T2D; *LEP* was higher expressed in patients with dyslipidemia, while *PECAM1* showed lower expression in the same patients; moreover *IL6* and *TNFA* were higher expressed in patients with chronic

periodontitis. Furthermore, data analysis suggests that elevated levels of mainly pro-inflammatory cytokines, such as IL-6, TNF-α, LEP and RETN, are correlated and associated simultaneously with worse lipid and glycemic parameters and with periodontal clinical parameters that indicate the severity of chronic periodontitis. These findings reinforce the evidence that a hyper-inflammatory status connect systemic and oral inflammatory diseases, such as type 2 diabetes, dyslipidemia and chronic periodontitis. Further case-control studies and meta-analysis approach studying these three diseases simultaneously should be made to confirm and strengthen our results.

Author contributions

Rafael Nepomuceno, Silvana R. Perez Orrico and Raquel M. Scarel-Caminaga – Conceptualization;

Raquel M. Scarel-Caminaga - Data curation;

Rafael Nepomuceno, Bruna de Freitas Vallerini, Sâmia Cruz Tfaile Corbi and Alliny de Souza Bastos - Formal analysis; Silvana R. Perez Orrico and Raquel M. Scarel-Caminaga - Funding acquisition;

Bruna de Freitas Vallerini, Rafael Nepomuceno, Romerito Lins da Silva, Sâmia Cruz Tfaile Corbi and Alliny de Souza Bastos – Investigation;

Rafael Nepomuceno, Bruna de Freitas Vallerini, Romerito Lins da Silva, Sâmia Cruz Tfaile Corbi and Alliny de Souza Bastos – Methodology;

Raquel Alves dos Santos, Catarina Satie Takahashi, Raquel M. Scarel-Caminaga; Resources, Raquel Alves dos Santos, Catarina Satie Takahashi, Raquel M. Scarel-Caminaga - Project administration;

Rafael Nepomuceno, Silvana R. Perez Orrico and Raquel M. Scarel-Caminaga; Writing – original draft.

Raquel Alves dos Santos, Catarina Satie Takahashi, Silvana R. Perez Orrico and Raquel M. Scarel-Caminaga – Supervision;

Raquel Alves dos Santos, Catarina Satie Takahashi, Silvana R. Perez Orrico and Raquel M. Scarel-Caminaga - Writing – review & editing.

Conflict of interest

The authors declare that they have no competing interests.

Data availability statement

The data that support the findings of this study are available from the corresponding author, RMSC, upon reasonable request.

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