Research Article

Autoantibodies to the β_1 -Adrenoceptor from Patients with Periodontitis as a Risk Factor for Cardiac Dysfunction

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The presence of serum autoantibodies in periodontitis (P) patients against β_1 -adrenoceptor (β_1 -AR), using cardiac membranes or a synthetic β_1 -AR peptide corresponding to the second extracellular loop of human β_1 -AR as antigens, permit us to detect circulating antibody from 40 P patients but not in 20 normal individuals (control). Simultaneously, the P patients exhibited a decrease in HRV. Anti- β_1 -AR IgG titers correlated with the decrease in HRV of the same patients and the anti- β_1 -AR peptide IgG displayed partial agonist-like activity and modified the contractility of isolated atria, produced cyclic nucleotides, and inhibited the β_1 -AR agonistic activity of isoproterenol. We demonstrated in this study an association between periodontitis infection and an increased risk of cardiac disease, thereby highlighting the role of anti- β_1 -AR autoantibodies in alteration of myocardial contractility.

1. Introduction

Cardiovascular disease occurs as a result of a complex set of genetic and environmental factors [1]. Although the identity of the traditional risk factors has been established, they do not fully account for the risk of cardiovascular disease, suggesting that other mechanisms contribute to the pathogenic process [2]. Recently, several lines of evidence have implicated chronic inflammation in the etiology of cardiovascular disease [3].

Periodontitis is an inflammatory disease of tooth-supporting structures that leads to the destruction of connective tissue, loss of periodontal attachment, and resorption of alveolar bone [4].

The pathogenesis of the disease involves essential immunological factors associated with infections caused by bacteria into subgingival plaque [5]. Among the immunological factors, local synthesis of biological products (e.g., enzymes produced by cells of the supporting tissue) as well as bacterial-specific secretion of immunoglobulin (Ig), soluble inflammatory mediators, and/or cellular/tissue degradation products has been described [6–9]. It has been shown that endotoxins of plaque microorganisms penetrate gingival

tissues and enter the bloodstream in sufficient numbers to cause a systemic lipopolysaccharide-specific antibody response [10].

However, in periodontal disease during the process of combating bacterial infection, the immune system may cause localized tissue damage and activate the systemic humoral immune response [11, 12]. Thus, the susceptibility to, and progression of, the disease is dependent upon not only the virulence of the microbial pathogens, but also the host response to regulation of tissue destruction.

Initial studies of autoimmunity in the pathogenesis of periodontal disease focused on detection of autoantibodies directed towards various self-antigens [13–15], such as autoantibodies against human gingival fibroblasts [16, 17]. Specifically, these antigingival fibroblast autoantibodies could interact with β_1 -ARs exhibiting adrenergic agonistic activity.

Moreover, autoantibodies with immune reactivity against β_1 -ARs of the myocardium have been described in the sera of patients with different cardiomyopathies associated with infection with parasites and viruses [18–22]. Increasing evidence indicates that periodontal disease is a risk factor for cardiovascular disease and stroke [23, 24]. The main

underlying pathological pathway for cardiomyopathy associated with periodontal disease is atherosclerosis [2]. Increasing evidence suggests that the progression of atherosclerosis arises from a combination of dysfunction and inflammation of the endothelium. Inflammatory mediators derived from infected periodontal tissue or periodontopathic bacteria (or their products) could induce dysfunction of endothelial cells [2].

We demonstrated that serum autoantibodies from patients with periodontitis could react with the myocardial surface and with human β_1 -ARs. A correlation between the serum antibodies against myocardial β_1 -ARs and alteration in heart rate variability (HRV) could be determined. The capacity of β_1 -AR autoantibodies to modify atria contractility *in vitro* was also assessed.

2. Patients and Methods

2.1. Patients. The study group consisted of 40 male patients (mean age: 41 years; range: 32–50 years) with periodontitis attending periodontology clinics in the metropolitan area of Buenos Aires. Twenty healthy male subjects (mean age: 38 years; range: 30–46 years) were used as controls. The characteristic signs of periodontitis included the following: loss of clinical attachment; horizontal or/and angular alveolar bone loss; periodontal pocket formation; gingival inflammation. To be included in the study, at least six sites with ongoing periodontal disease were required. Measurements in patients with periodontitis included sites with alveolar bone loss >2 mm and pocket depth >5 mm with bleeding and attachment loss >3 mm. In healthy subjects (control group), the probing depth was <3 mm and attachment loss was <2 mm. None of the subjects (patients in group I and controls in group II) had systemic illnesses and they were not smokers. Patients had not received periodontal treatment or antibiotics within the preceding 5 months or anti-inflammatory drugs 3 weeks before the study. Also, it can be seen in Tables 1 and 2 the general characteristic of the study populations and selection index. All patients consented to participate in the study. The investigation was conducted according to the tenets of the Declaration of Helsinki.

2.2. HRV. The 20 patients with periodontitis and 20 normal subjects were studied according to a previous report [25]. Heart rate was recorded using an Oxford Medilog 2–24 miniature analog tape recorder (Oxford Instruments Medical Systems, Abingdon, UK). The recording speed was 2 mms⁻¹. HRV was assessed from RR-interval files generated from 10 pm to 8 am analyzed in hourly epochs. The summary time-domain statistical measures of HRV calculated included the HRV long-term standard deviation of all NN (SDNN) indices. These indices represent variability over heart cycles and estimate high-frequency variation in the heart rate [25].

2.3. Human Sera and IgG Purification. Sera and the corresponding IgG were obtained from patients with periodontitis (group I) and from normal individuals (group II). Six milliliters of blood were obtained by venipuncture. Blood

samples were allowed to clot at room temperature. The serum was separated by centrifugation at 2000 × g and stored at –20°C until used in assays. IgG was obtained by precipitation followed previous reports [17, 18].

2.4. Purification of Anti-peptide Antibodies by Affinity Chromatography. The IgG fraction of group-I patients was independently subjected to affinity chromatography on the synthesized peptide covalently linked to Affi-Gel 15 (Bio-Rad, Richmond, CA, USA). The IgG fraction was loaded on the affinity column equilibrated with phosphate-buffered saline (PBS) according to previous reports [16, 17].

2.5. Membrane Preparation. Cardiac membranes from male rat Wistar strain were prepared as previously described [17, 18]. In brief, cells or atria were homogenized in an Ultra-Turrax homogenizer (IKA, Wilmington, NC, USA) at 4°C in 6 volumes of potassium phosphate buffer, 1 mM MgCl₂, 0.25 M sucrose (pH 7.5) supplemented with 0.1 mM phenylmethyl sulphonyl fluoride (PMSF), 1 mM ethylenediamine tetra-acetic acid (EDTA), 5 µg mL⁻¹ leupeptin, 1 µM bacitracin, and 1 µM pepstatin A. The homogenate was centrifuged twice for 10 min at 3000 × g, then at 10,000 × g and 40,000 × g at 4°C for 15 min and 90 min, respectively. The resulting pellets were suspended in 50 mM phosphate buffer fortified with the same protease inhibitors (pH 7.5).

2.6. ELISA Assay. Fifty micromiliters of peptide solution at 50 µg/mL concentration or purified cardiac membranes (50 µg/mL) in 0.1 M Na₂CO₃ buffer (pH 9.6) were used to coat microtiter plates at 4°C overnight. After blocking the wells with 2% bovine serum albumin in PBS for 1 h at 37°C, 10 µL of a 1/30 dilution of sera of different concentrations or purified IgG from groups I and II were added in triplicate and allowed to react with the peptide for 2 h at 37°C. After thoroughly washing the wells with 0.05% Tween 20 in PBS, 100 µL of 1:6000 goat anti-human IgG alkaline phosphate conjugate antibodies were added and incubated for 1 h at 37°C. After extensive washing, *p*-nitrophenylphosphate (1 mg/mL) was added as the substrate and the reaction stopped after 30 min. Optical density (OD) was measured at 405 nm with an ELISA reader. As a negative control, nonantigen paired wells with M₁ cholinergic peptide and wells with no primary antiserum were also used. The results for each sample were expressed as the mean ± standard error of the mean (s.e.m.) of triplicate values.

2.7. Contractile Study. Male rats Wistar strain (200–250 g) were decapitated and atria quickly removed. Atria were placed in a glass chamber containing Krebs–Ringer bicarbonate (KRB) solution (pH 7.4) that was gassed with 5% CO₂ in oxygen at 30°C. After a stabilization period of 30 min, spontaneous tension and frequency were recorded using a force transducer coupled to an ink-writing oscillograph, as previously described [18].

2.8. Assays for Cyclic Adenosine Monophosphate (cAMP) and Cyclic Guanosine Monophosphate (cGMP). Rat atria (10 mg)

TABLE 1: Characteristic of the study populations.

Demography and risk factors	Periodontitis patients group I ($n = 40$)	Healthy subjects group II ($n = 20$)
Gender		
Male	36	18
Female	4	2
Education level		
Elementary school	38	18
High school	2	2
BMI (range kg/m^2)	From 20 to 25	From 19 to 22
Measure blood pressure (mmHg)		
Measure SBP (mean $\pm$ SD)	138 ± 20	118 ± 14
Measure DBP (mean $\pm$ SD)	87 ± 12	75 ± 10
Laboratory examination		
Cholesterol total (mean $\pm$ SD), mg/dl	170 ± 21	176 ± 22
LDL (mean $\pm$ SD), mg/dl	129 ± 14	130 ± 21
HDL (mean $\pm$ SD), mg/dl	34 ± 10	32 ± 11

BMI: Body Mass Index (range $\geq 27 \text{ kg/m}^2$); SBP: Systolic Blood Pressure. DBP: Diastolic Blood Pressure; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

TABLE 2: Periodontitis selection index.

Parameters	Numerical ranges
PPD	$\geq 6 \text{ mm}$
CAL	$\geq 6 \text{ mm}$

PPD: pocket probing depth; CAL: clinical attachment loss.

were incubated in 1 mL KRB for 30 min and the β_1 agonist isoproterenol (ISO) added in the last 15 min. When blockers were used, they were added 25 min before the addition of ISO. After incubation, atria were homogenized in 2 mL of absolute ethanol and centrifuged at $6000 \times g$ for 15 min at 4°C . Pellets were then rehomogenized in ethanol-water (2:1). Supernatants were collected and evaporated to dryness as described above. Cyclic AMP or cyclic GMP in the residue was dissolved in $400 \mu\text{L}$ of 0.05 M sodium acetate buffer (pH 6.2). For determination of nucleotides, we used ELISA employing the protocol of production of cAMP and cGMP from Amersham Biosciences (Piscataway, NJ, USA). Results were expressed in pico moles per milligram of wet weight of tissue (pmol/mg tissue ww).

2.9. Drugs. Stock solutions of ISO and atenolol were freshly prepared before each experiment. The β_1 peptide corresponded to the sequence of the second extracellular loop of the human β_1 -AR (HWWRA ESDEA RRCYN DPKCC DFVTN RC). An unrelated peptide derived from the second extracellular loop of the human M_2 cholinoreceptor (VRTVE DGEYC IQFFS NAAVT FGTA) was used as a control. Radioactive material, synthetic peptides, and β -adrenergic antagonists were from Dupont/New England Nuclear (Boston, MA, USA), Sigma Genosys (St. Louis, MO, USA), and Sigma-Aldrich (St. Louis, MO, USA), respectively.

2.10. Statistical Analysis. The Student's t -test for unpaired values was used to determine the levels of significance. Analysis of variance (ANOVA) and a *post hoc* test (Dunnett's method and Student-Newman-Keuls test) were employed when pairwise multiple comparison procedures were necessary. Differences between means were considered significant if $P < 0.05$.

3. Results

ELISA assays were carried out to demonstrate if there was a correlation between the level of IgG in the serum of patients with periodontitis and cardiac membranes and the β_1 -AR. Figure 1 shows the OD values for sera from patients with periodontitis (group I) and normal subjects (group II) using rat cardiac membrane (a) and β_1 -AR synthetic peptide (b) as coating antigens. The OD values obtained from group-I sera were always more than two standard deviations ($>2 \text{ SD}$) from those of normal individuals. Figure 1(c) shows a positive correlation between serum anticardiac membrane and anti- β_1 -AR peptide antibodies of the individual sera from group I. It was noted that 34/40 (85%) and 32/40 (80%) of patients' sera reacted positively against cardiac membranes and β_1 -AR synthetic peptide, respectively. When unrelated synthetic peptide (M_2 cholinoreceptor peptide) was used as a coating antigen, IgG from group I and from group II gave negative results (data not shown).

Knowing that the β_1 -AR is involved in regulating various cardiac parameters (including heart rate and HRV), we evaluated the HRV in 20 patients with periodontitis whose sera reacted positively against β_1 synthetic peptide and in 20 normal individuals who acted as controls. The differences between patients with periodontitis and normal subjects with respect to the SDNN index are shown in Figure 2(a).

Number of XY Pairs	40
Pearson <i>r</i>	0.773
95 (%) confidence interval	0.6078 to 0.8741
<i>P</i> value (two tailed)	<i>P</i> < 0.0001
<i>P</i> value summary	***
Is the correlation significant? ($\alpha = 0.05$)	Yes
<i>R</i> squared	0.5975

FIGURE 1: Immunoreactivity of circulating IgG antibodies against cardiac membrane (a) or against β_1 -AR synthetic peptide (b). The optical density (OD) for serum sample (1/30 dilution) from 40 periodontitis patients (group I) or 20 normal individuals (group II) were evaluated by duplicate. Values are mean $\pm$ SD. Cutoff values of OD 0.180 and 0.210 for cardiac membranes and anti- β_1 -AR synthetic peptide, respectively. Correlation (c) between titers of serum anti- β_1 -AR peptide IgG and anticardiac membrane IgG from periodontitis patients. Anti- β_1 -AR peptide IgG titers were plotted as function of anti cardiac membranes. The values correspond to 40 periodontitis patients.

Number of XY Pairs	20
Pearson r	-0.7289
95 (%) confidence interval	-0.8656 to -0.4227
P value (two tailed)	0.0003
P value summary	***
Is the correlation significant? ($\alpha = 0.05$)	Yes
R squared	0.5313

FIGURE 2: (a) Heart rate variability long-term standard deviation (SDNN) index in 20 patients with periodontitis (group I) and in 20 normal individuals (group II). (b) Correlation between values of the SDNN index and titers of serum anti- β_1 -AR peptide IgG. The SDNN index was plotted as a function of serum titers of anti- β_1 -AR peptide IgG corresponding to the same patients.

TABLE 3: Influence of β_1 blockade on the stimulatory and inhibitory effects of anti- β_1 -AR peptide IgG.

Addition	dF/dt	cAMP	cGMP
Basal	1.8 ± 0.21	0.38 ± 0.03	0.041 ± 0.004
β_1 -AR IgG (5×10^{-8} M)	$3.9 \pm 0.32^*$	$1.25 \pm 0.11^*$	0.051 ± 0.004
β_1 -AR IgG (5×10^{-8} M) + atenolol	$2.1 \pm 0.19^{**}$	$0.41 \pm 0.04^{**}$	—
β_1 -AR IgG + β_1 -AR peptide	$2.3 \pm 0.23^{**}$	$0.42 \pm 0.03^{**}$	—
Normal IgG (5×10^{-8} M)	1.9 ± 0.18	0.40 ± 0.02	0.039 ± 0.04
Basal	2.1 ± 0.22	0.40 ± 0.03	0.042 ± 0.004
β_1 -AR IgG (5×10^{-7} M)	$0.6 \pm 0.05^*$	$0.21 \pm 0.02^*$	$0.350 \pm 0.03^*$
β_1 -AR IgG (5×10^{-7} M) + atenolol	$1.7 \pm 0.15^{**}$	$0.38 \pm 0.03^{**}$	$0.122 \pm 0.02^{**}$
β_1 -AR IgG + β_1 -AR peptide	$1.9 \pm 0.20^{**}$	$0.37 \pm 0.02^{**}$	$0.101 \pm 0.02^{**}$
Normal IgG (5×10^{-7} M)	2.0 ± 0.19	0.42 ± 0.03	0.043 ± 0.003

Values are mean $\pm$ s.e.m. of ten patients with periodontitis in each group carried out in duplicate. Contractility (dF/dt: g/s), and levels of cAMP (pmol/mg tissue ww) and cGMP (pmol/mg tissue ww) were measured after incubation for 15 min with rat atria in the presence of anti- β_1 -AR peptide IgG (β_1 -AR IgG) and normal IgG or in the absence (basal) of IgG. Atenolol (5×10^{-7} M) and β_1 -AR peptide (5×10^{-5} M) were added before the IgG. * $P < 0.001$ versus basal; ** $P < 0.001$ versus β_1 -AR IgG alone.

There was a significant decrease in HRV (mean $\pm$ SD: 65.1 ± 9.2) in patients with periodontitis in comparison with the control group (mean $\pm$ SD: 90.3 ± 10). When the SDNN index was plotted as a function of serum titers, anti- β_1 -AR peptide IgG from each patient with periodontitis showed a negative correlation between serum anti- β_1 -AR peptide antibodies and the SDNN index (Figure 2(b)).

We observed that patients with periodontitis showed a profound alteration in cardiac contractility *in vivo*, so we wanted to ascertain if serum anti- β_1 -AR antibodies could trigger cardiac β_1 -AR functional alterations in atria. For this purpose, we selected the 20 patients with periodontitis who showed a decrease in HRV and whose sera were positive against β_1 -adrenergic synthetic peptide in the ELISA assay. The IgG fraction was independently subjected to affinity chromatography and eluted from the column covalently linked to β_1 -adrenergic synthetic peptide (anti- β_1 -AR peptide IgG). Increasing concentrations of anti- β_1 -AR peptide IgG were applied to isolated spontaneously beating rat atria and changes in the magnitude of contractility (dF/dt) measured (Figure 3).

Anti- β_1 -AR peptide IgG behaved as a partial β_1 -adrenergic agonist; increasing dF/dt at low concentrations and at higher concentrations, autoantibodies decreased dF/dt (Figures 3(a) and 3(b)). The stimulatory and inhibitory effects were accompanied by modification of cAMP production (Figure 3(a)) and cGMP production (Figure 3(b)). cAMP production correlated positively with the stimulatory effect of the antibodies (Figure 3(c)), but cGMP production correlated negatively with the inhibitory effect of the antibodies (Figure 3(d)). Table 3 shows the effect of blockade upon the effect of IgG on dF/dt, cAMP and cGMP. For the β_1 -AR, the stimulatory and inhibitory effects were almost abolished by pretreatment of atria with 1×10^{-7} M atenolol (β_1 -AR antagonist) and by 1×10^{-5} M β_1 -adrenergic synthetic peptide. The IgG from normal subjects and the nonpeptide fraction gave negative results (data not shown).

To ascertain if anti- β_1 -AR peptide IgG interacted with the same cardiac β_1 -AR epitope as an authentic agonist (ISO), we

studied the action of anti- β_1 -AR peptide antibody upon ISO. The maximal positive effects of 5×10^{-8} M ISO on dF/dt (g/s: 5.3 ± 0.5 , $n = 5$) and the cAMP stimulation (pmol/mg tissue ww: 2.8 ± 0.3 , $n = 5$) were blunted by 5×10^{-7} M anti- β_1 -AR peptide IgG.

4. Discussion

We previously demonstrated that patients with periodontitis have functional serum IgG that interacts with the β_1 -AR of gingival fibroblasts. In the present study, we confirmed the presence of anti- β_1 -AR autoantibodies in the sera of patients with periodontitis and provided evidence that these sera can also recognize the cardiac membranes of rats. Most of the sera that reacted positively against rat cardiac membranes showed positive immune reactivity to human β_1 -AR synthetic peptide. Furthermore, a significant correlation between anticardiac membrane antibodies and anti- β_1 -AR peptide IgG was observed.

Using a synthetic peptide with an identical amino acid sequence of the second extracellular loop of human β_1 -AR, we established that the β_1 -AR is a target for the anticardiac autoantibodies described in patients with periodontitis. Due to the strong homology between rodent and human β_1 -AR peptides [26], one can assume that this is the one recognized by the autoantibodies. It has been shown that the second extracellular loop of cardiovascular G-protein-coupled receptors is an antigenic target for the generation of autoantibodies in patients with cardiomyopathy [27]. Therefore, we studied the β_1 -AR-mediated effect of autoantibodies on isolated rat atria using affinity-purified anti- β_1 -AR peptide IgG from patients with periodontitis.

HRV is a powerful predictor of cardiovascular mortality in healthy people, those who have had a myocardial infarction, and in patients with heart failure. An elevated resting heart rate, reduced HRV, or both are negative prognostic factors independent of other clinical parameters, including left ventricular function [28]. Impaired control of HRV is linked to a deleterious sympathovagal imbalance

Number of XY Pairs	5
Pearson <i>r</i>	0.9988
95 (%) confidence interval	0.9815 to 0.9999
<i>P</i> value (two tailed)	<i>P</i> < 0.0001
<i>P</i> value summary	***
Is the correlation significant? (α =0.05)	Yes
<i>R</i> squared	0.9977

Number of XY Pairs	5
Pearson <i>r</i>	-0.9625
95 (%) confidence interval	-0.9969 to -0.7529
<i>P</i> value (two tailed)	0.0028
<i>P</i> value summary	**
Is the correlation significant? (α =0.05)	Yes
<i>R</i> squared	0.9654

FIGURE 3: Effect of increasing concentrations of anti-β₁-AR peptide IgG (IgG) on contractility (dF/dt) and production of cAMP and cGMP in isolated atria from rats. (a) Comparative effects of anti-β₁-AR peptide IgG on dF/dt (●-●) and on cAMP production (○-○). (b) Comparative effects of anti-β₁-AR peptide IgG on dF/dt (●-●) and on cGMP production (○-○). (c) Positive correlation between cAMP and dF/dt (corresponding data to A values). (d) Negative correlation between cGMP and dF/dt (corresponding data to B values). Values are mean ± s.e.m. of 20 patients with periodontitis.

characterized by predominance of the sympathetic nervous system [28].

In the present study, the finding of a correlation between decrease in HRV and the presence of serum anticardiac membrane autoantibodies and anti- β_1 -AR autoantibodies in patients with periodontitis allows us to suggest that the lower HRV observed in such patients is due to fixation of antibodies upon receptors. HRV alteration may be a consequence of activation of the β_1 -AR induced by antibody β_1 -AR interaction on the cardiac sarcolemma.

We also provide evidence that isolated fractions of IgG enriched in anti- β_1 -AR peptide antibody could display partial agonistic activity on contractility in isolated atria. The partial agonistic activity of autoantibodies observed *in vitro* mimics the action on HRV of the partial β_1 -adrenergic agonist celiprolol described *in vivo*. However, the combination of celiprolol with atenolol did not prevent the fall in HRV triggered by the partial agonist [25]. Hence, the antibody-mediated partial β_1 agonistic activity could be responsible for the decrease in HRV in patients with periodontitis.

The increase in contractility observed at low concentrations of IgG correlated with the increase in production of cAMP, whereas the decrease in contractility triggered by higher concentrations of IgG correlated with an increase in cGMP production. The increase and decrease in contractility are related to β_1 -AR function because they were blunted by atenolol. The increase in cGMP production which could limit the increase in contractility of β -adrenergic stimulation of isolated rat atria has been established [29].

The interaction of anti- β_1 -AR autoantibodies with the receptor has two functional implications: (i) it directly modifies the sympathetic activity of the myocardium; (ii) it decreases the effectiveness of the authentic agonist ISO. This raises the question: could there be a common explanation for the abnormal sympathetic activity in the cardiovascular system mediated by cardiac β_1 -AR autoantibodies? If so, antibody fixation could increase sympathetic activity for an extended period and result in a chronically elevated heart rate. Simultaneously, the antagonistic activity of the autoantibodies could lead to deleterious sympathovagal imbalance, resulting in functional deregulation associated with pathologic remodelling, myocyte apoptosis, and alteration of calcium handling that leads to myocardial ischemia, a decrease in contractile function, and an increased risk of ventricular arrhythmias [30].

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Conflict of Interests

There are no competing interests.

References

- [1] K. Karnoutsos, P. Papastergiou, S. Stefanidis, and A. Vakiloudi, "Periodontitis as a risk factor for cardiovascular disease: the role of anti-phosphorylcholine and anti-cardiolipin antibodies," *Hippokratia*, vol. 12, no. 3, pp. 144–149, 2008.
- [2] T. Nakajima and K. Yamazaki, "Periodontal disease and risk of atherosclerotic coronary heart disease," *Odontology*, vol. 97, no. 2, pp. 84–91, 2009.
- [3] L. L. Humphrey, R. Fu, D. I. Buckley, M. Freeman, and M. Helfand, "Periodontal disease and coronary heart disease incidence: a systematic review and meta-analysis," *Journal of General Internal Medicine*, vol. 23, no. 12, pp. 2079–2086, 2008.
- [4] H. Toker, I. Marakoglu, and O. Poyraz, "Effect of meloxicam on gingival crevicular fluid IL-1beta and IL1 receptor antagonist levels in subjects with chronic periodontitis, and its effects on clinical parameters," *Clinical Oral Investigations*, vol. 10, no. 4, pp. 305–310, 2006.
- [5] M. A. Listgarten and P. M. Loomer, "Microbial identification in the management of periodontal diseases. A systematic review," *Annals of Periodontology*, vol. 8, no. 1, pp. 182–192, 2003.
- [6] J. Gamonal, A. Acevedo, A. Bascones, O. Jorge, and A. Silva, "Levels of interleukin-1 β , -8, and -10 and RANTES in gingival crevicular fluid and cell populations in adult periodontitis patients and the effect of periodontal treatment," *Journal of Periodontology*, vol. 71, no. 10, pp. 1535–1545, 2000.
- [7] A. Holmlund, L. Hnstrm, and U. H. Lerner, "Bone resorbing activity and cytokine levels in gingival crevicular fluid before and after treatment of periodontal disease," *Journal of Clinical Periodontology*, vol. 31, no. 6, pp. 475–482, 2004.
- [8] P. A. Heasman, J. G. Collins, and S. Offenbacher, "Changes in crevicular fluid levels of interleukin-1 beta, leukotriene B4, prostaglandin E2, thromboxane B2 and tumour necrosis factor alpha in experimental gingivitis in humans," *Journal of Periodontal Research*, vol. 28, no. 4, pp. 241–247, 1993.
- [9] I. B. Lamster, R. L. Oshrain, R. S. Celenti, J. B. Fine, and J. T. Grbic, "Indicators of the acute inflammatory and humoral immune responses in gingival crevicular fluid: relationship to active periodontal disease," *Journal of Periodontal Research*, vol. 26, no. 3, pp. 261–263, 1991.
- [10] E. Hernichel-Gorbach, K. S. Kornman, S. C. Holt et al., "Host responses in patients with generalized refractory periodontitis," *Journal of Periodontology*, vol. 65, no. 1, pp. 8–16, 1994.
- [11] C. A. Ramseier, J. S. Kinney, A. E. Herr et al., "Identification of pathogen and host-response markers correlated with periodontal disease," *Journal of Periodontology*, vol. 80, no. 3, pp. 436–446, 2009.
- [12] H. A. Schenkein, "Host responses in maintaining periodontal health and determining periodontal disease," *Periodontology 2000*, vol. 40, no. 1, pp. 77–93, 2006.
- [13] C. G. Dileep Sharma and A. R. Pradeep, "Anti-neutrophil cytoplasmis autoantibodies: a renewed paradigm in periodontal disease pathogenesis?" *Journal of Periodontology*, vol. 77, no. 8, pp. 1304–1313, 2006.
- [14] P. S. Rajapakse and A. E. Dolby, "Evidence for local production of antibodies to auto and non-self antigens in periodontal disease," *Oral Diseases*, vol. 10, no. 2, pp. 99–105, 2004.
- [15] L. A. De-Gennaro, J. D. Lopes, and M. Mariano, "Autoantibodies directed to extracellular matrix components in patients with different clinical forms of periodontitis," *Journal of Periodontology*, vol. 77, no. 12, pp. 2025–2030, 2006.

- [16] L. Sterin-Borda, C. Furlan, and E. Borda, "Circulating beta 1 adrenergic auto antibodies from patients with chronic periodontitis interact with gingival fibroblasts," *European Journal of Dentistry*, vol. 3, pp. 191–199, 2009.
- [17] L. Sterin-Borda, C. Furlan, and E. Borda, "Autoantibodies to β 1-adrenoceptors in human chronic periodontitis induce overexpression of fibroblast CD40 and trigger prostaglandin E2 generation," *Journal of Periodontal Research*, vol. 44, no. 3, pp. 330–337, 2009.
- [18] L. Sterin-Borda, G. Gorelik, M. Postan, S. Gonzalez Cappa, and E. Borda, "Alterations in cardiac beta-adrenergic receptors in chagasic mice and their association with circulating beta-adrenoceptor-related autoantibodies," *Cardiovascular Research*, vol. 4, no. 4, pp. 116–125, 1970.
- [19] L. Joensen, E. Borda, T. Kohout, S. Perry, G. García, and L. Sterin-Borda, "*Trypanosoma cruzi* antigen that interacts with the β 1-adrenergic receptor and modifies myocardial contractile activity," *Molecular and Biochemical Parasitology*, vol. 127, no. 2, pp. 169–177, 2003.
- [20] L. Sterin-Borda and E. Borda, "Role of neurotransmitter autoantibodies in the pathogenesis of chagasic peripheral dysautonomia," *Annals of the New York Academy of Sciences*, vol. 917, pp. 273–280, 2000.
- [21] E. Borda, J. Pascual, and P. Cossio, "A circulating IgG in Chagas' disease which binds to β -adrenoceptors of myocardium and modulates their activity," *Clinical and Experimental Immunology*, vol. 57, no. 3, pp. 679–686, 1984.
- [22] C. J. Limas, I. F. Goldenberg, and C. Limas, "Autoantibodies against β -adrenoceptors in human idiopathic dilated cardiomyopathy," *Circulation Research*, vol. 64, no. 1, pp. 97–103, 1989.
- [23] W. Nesse, P. U. Dijkstra, F. Abbas et al., "Increased prevalence of cardiovascular and autoimmune diseases in periodontitis patients: a cross-sectional study," *Journal of Periodontology*, vol. 81, no. 11, pp. 1622–1628, 2010.
- [24] G. R. Persson and R. E. Persson, "Cardiovascular disease and periodontitis: an update on the associations and risk," *Journal of Clinical Periodontology*, vol. 35, no. 8, pp. 362–379, 2008.
- [25] B. Silke and J. G. Riddell, "Heart rate variability effects of an agonist or antagonists of the β -adrenoceptor assessed with scatterplot and sequence analysis," *Clinical Autonomic Research*, vol. 8, no. 3, pp. 145–153, 1998.
- [26] J. R. Jasper, R. E. Link, A. J. Chruscinski, B. K. Kobika, and B. Berstein, "Primary structure of the mouse β 1-adrenergic receptor gene," *Biochimica et Biophysica Acta*, vol. 178, pp. 307–309, 1993.
- [27] M. L. X. Fu, W. Schulze, G. Wallukat, A. Hjalmarson, and J. Hoebeke, "Functional epitope analysis of the second extracellular loop of the human heart muscarinic acetylcholine receptor," *Journal of Molecular and Cellular Cardiology*, vol. 27, no. 1, pp. 427–436, 1995.
- [28] D. Feldman, T. S. Elton, D. M. Menachemi, and R. K. Wexler, "Heart rate control with adrenergic blockade: clinical outcomes in cardiovascular medicine," *Vascular Health and Risk Management*, vol. 6, pp. 387–397, 2010.
- [29] L. Sterin-Borda, A. Genaro, C. P. Leiros, G. Cremaschi, A. V. Echagüe, and E. Borda, "Role of nitric oxide in cardiac β -adrenoceptor-inotropic response," *Cellular Signalling*, vol. 10, no. 4, pp. 253–257, 1998.
- [30] M. J. De Jong and D. C. Randall, "Heart rate variability analysis in the assessment of autonomic function in heart failure," *The Journal of Cardiovascular Nursing*, vol. 20, no. 3, pp. 186–197, 2005.