Nitric Oxide Synthase in Gingival Tissues of Patients With Chronic Periodontitis and With and Without Diabetes

Zeynep Pan,* Esra Guzeldemir,* Hilal Uslu Toygar,* Nebil Bal,† and Sule Bulut*

**Background:** The purpose of this study was to evaluate the expression of inducible nitric oxide synthase (iNOS) in the gingival tissues of periodontitis patients with and without type 2 diabetes to assess whether NO plays a role in the severity of periodontitis in patients with diabetes. Patients with diabetes and healthy patients were used as controls.

**Methods:** A total of 80 patients were evaluated in four groups (with 20 subjects each): patients with chronic periodontitis and diabetes (12 males and eight females; mean age, 52.1 ± 6.9 years), patients with chronic periodontitis who were otherwise healthy (12 males and eight females; mean age, 43.1 ± 8.9 years), periodontally healthy patients with diabetes (12 males and eight females; mean age 50.9 ± 6.3 years), and systemically and periodontally healthy control subjects (12 males and eight females; mean age 29.8 ± 9.2 years). Periodontal parameters were recorded. Immunohistochemistry was used to detect inflammation and iNOS expression in gingival tissues.

**Results:** Although periodontal parameters were slightly higher in periodontitis compared to diabetic periodontitis, immunohistochemical parameters were higher in diabetic periodontitis compared to periodontitis. All periodontal parameters were higher in patients with periodontitis and with/without diabetes compared to controls and patients with diabetes. All immunohistochemical parameters were higher in patients with diabetes and periodontitis compared to patients with only diabetes or periodontitis, but there was no difference between the latter two groups. There was a correlation between the expression of iNOS and inflammatory cells in controls, patients with diabetes, and patients with periodontitis but not in patients with diabetes and periodontitis.

**Conclusions:** Inflammation and iNOS expression were more prominent in the gingiva of the patients with both diabetes and periodontitis. However, iNOS expression did not seem to have an additional detrimental effect on the course of periodontitis in patients with diabetes compared to those with periodontitis alone.

**KEY WORDS**
Chronic periodontitis; diabetes mellitus; gingiva; nitric oxide; nitric oxide synthase.

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Diabetes mellitus is a highly prevalent metabolic disorder; 150 million people had the disease in the year 2000, and it is expected to increase to 220 million by 2010. The prevalence of diabetes in the Turkish population is 7.2%. Diabetes has two main forms: type 1 diabetes mellitus and type 2 diabetes mellitus. The more common form, type 2 diabetes mellitus (DM), results from insulin resistance (i.e., impaired insulin effectiveness) and the failure of pancreatic beta cells to produce sufficient insulin. The subsequent hyperglycemia has wide-ranging molecular and cellular effects, resulting in oxidative stress, upregulation of proinflammatory responses, and vascular changes that predispose individuals to the classic diabetes complications of cardiovascular disease, nephropathy, retinopathy, neuropathy, and atherosclerosis.

Periodontal disease has been termed the sixth complication of diabetes; diabetes also is an important risk factor for periodontal diseases. Wang et al. found in an epidemiologic study that the prevalence of periodontal disease was ~10% higher in subjects with diabetes than in those without diabetes. It was shown that individuals with...
Nitric Oxide Synthase in Periodontitis Patients With Diabetes

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The relationship between periodontitis and diabetes is bidirectional,18 insofar as the presence of one condition tends to promote the other. The meticulous management of either may assist the treatment of the other.19 Diabetes does not result in gingivitis or periodontal defects, per se, but it alters the response of the periodontal tissues to local pathogenic factors, causing a reduction in defense mechanisms and an increased susceptibility to infection. The explanation for the increased risk and severity of periodontitis in diabetes is primarily provided by a number of cellular and molecular alterations taking place in the periodontium as a consequence of sustained hyperglycemia.20 Long-term hyperglycemia results in increased levels of advanced glycation end products (AGEs),21 enhanced susceptibility to infection due to diminished neutrophil recruitment and function,22 and a more severe inflammatory response that leads to greater tissue destruction.23

Hyperglycemia induces the non-enzymatic glycation and oxidation of proteins (e.g., collagen) and lipids, resulting in the accumulation of AGEs in diabetic tissues.24 The formation of AGEs, a critical link in many diabetic complications, also occurs in periodontium.24,25 The interaction of AGEs with endothelial cells increases oxidative stress, which has been associated with vascular injury. When the generation of reactive oxygen species (ROS), including superoxide anion (O$_2^-$), exceeds cellular defense mechanisms, these unstable molecules interact with biologic macromolecules, such as lipids, proteins, and DNA, and lead to structural changes as well as functional abnormalities, hence an increased ROS may contribute to diabetic complications.26 Oxidative stress has been considered a common pathogenic factor in diabetes and its complications.27

Periodontal diseases can induce or perpetuate an elevated systemic chronic inflammatory state, as reflected in increased serum C-reactive protein (CRP), interleukin (IL)-6 and -1β, tumor factor necrosis-alpha (TNF-α), and fibrinogen levels, and they may play a similar role to that of obesity in inducing or exacerbating insulin resistance.28 Elevation of proinflammatory cytokines caused by periodontitis may even predispose to the development of diabetes,29 and increased levels of inflammatory markers, such as CRP and IL-6, are reported to be significant risk indicators for DM. Moreover, the hemoglobin A1c (HbA1c) level deteriorated in patients with type 2 diabetes and severe periodontitis but not in patients without severe periodontitis.30

The prevention and treatment of periodontal diseases in patients with diabetes seems to have a beneficial effect on diabetes control.15,31-34 However, Promsudthi et al.17 found no such correlation.

Nitric oxide (NO), which is synthesized from L-arginine by NO synthase (NOS), plays a dual role in infection and the resulting inflammatory responses: it is a central mediator of host response and is an important agent in the pathogenesis of host damage.35 NOS exists in three distinct isoforms: neuronal NOS, endothelial NOS, and inducible NOS (iNOS). NOS and transformation determine the pathogenesis of periodontitis, which is mainly associated with inflammation.36 iNOS is produced by immunocompetent cells, such as macrophages infected with bacteria, and is involved in regulating inflammatory reactions37 induced by a variety of immunologic stimuli.38 Induction of iNOS occurs in response to bacterial lipopolysaccharide, inflammatory cytokines (e.g., interferon-gamma [IFN-γ], IL-1, and TNF-α), and the elements of the immune system (e.g., macrophages and leukocytes), and it produces a large amount of NO for a sustained time. Large quantities of NO have been associated with cell and tissue injury. NO exerts its harmful effects via direct cytotoxic or cytostatic actions.35 Because iNOS is expressed almost exclusively under inflammatory conditions, this has led to the hypothesis that iNOS has detrimental effects in inflamed tissues and promotes the inflammatory response.39 NO synthesis and iNOS activity were reported to be increased in inflamed periodontal tissues.40-45

Activity of the NO system in diabetes is controversial, with studies providing for an increase,46-48 a decrease,49,50 or no change.51,52 NO is an important vascular target for ROS. Superoxide neutralizes NO, and the peroxynitrite formed is a source of hydroxyl radicals that can cause endothelial damage. NO is released from the endothelium and plays an important role in the regulation of vascular tone, inhibits platelet aggregation, suppresses smooth muscle proliferation, and acts as an antiatherogenic factor. Diabetes constitutes one of the major independent cardiovascular risks, and patients with this disease have cardiovascular morbidity and mortality. Endothelial dysfunction due to reduced bioavailability of NO is an early step in the course of atherosclerotic cardiovascular disease.

There are no data concerning the pathogenetic role and contribution of iNOS in the inflammatory reactions of the periodontium in the course of diabetes. Hence, the aim of this study is to identify and evaluate...
the expression of iNOS in patients with periodontitis and diabetes to assess whether NO plays a role in the severity of periodontal disease in patients with type 2 diabetes.

MATERIALS AND METHODS
This study was conducted at the Periodontology Clinics, Adana Medical and Research Center, Baskent University, between September 2007 and August 2008. The study protocol was approved by the Ethics Committee of the Medical Faculty of Baskent University according to the Helsinki Declarations. Written informed consent was obtained after the completion of personal, medical, and dental questionnaires.

Study Population
A total of 80 subjects were recruited into the present cross-sectional study. Four study groups (n = 20 each) were created as follows: patients with DM and chronic periodontitis (CP) (DM + CP group; 12 males and eight females; mean age, 52.1 ± 6.9 years; range, 35 to 65 years), otherwise healthy patients with CP (CP group; 12 males and eight females; mean age, 43.1 ± 8.9 years; range, 39 to 60 years), periodontally healthy patients with DM (DM group; 12 males and eight females; mean age, 50.9 ± 6.3 years; range, 40 to 60 years), and systemically and periodontally healthy control subjects (C group; 12 males and eight females; mean age, 29.8 ± 9.2 years; range, 21 to 57 years). All subjects were recruited from Baskent University Adana Medical and Research Center Clinics.

All patients with diabetes were diagnosed as having DM ≥5 years prior to the study, using American Diabetes Association diagnostic criteria; all were under the supervision of an endocrinologist and were being treated with stable doses of oral hypoglycemic agents and/or insulin. Patients with diabetes were excluded if they had any known systemic diseases other than diabetes and a history of systemic or local disease with an influence on the immune system (cancer and cardiovascular and respiratory diseases), a history of hepatitis or human immunodeficiency virus infection, immunosuppressive chemotherapy, pregnancy or lactation, requirement for antibiotic prophylaxis, antibiotic therapy within the preceding 3 months or periodontal treatment within 6 months, or <20 teeth. To be included in the study, individuals had to be ≥18 years of age. Smokers were excluded from the study.

Glycemic Control
The fasting plasma glucose and the HbA1c test were used to monitor the overall glycemic control in patients known to have diabetes. The glycemic control was evaluated by the concentration of glycated HbA1c using high performance liquid chromatography.

Periodontal Examination
Whole-mouth clinical periodontal measurements were recorded at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, and mesio-lingual) and included plaque index (PI), gingival index (GI), PD, clinical attachment level (CAL), and dichotomous BOP score. A Williams periodontal probe was used for periodontal measurements.

Gingival Biopsy Collection and Analyses
Gingival biopsies of patients were taken under local anesthesia with 2% xylocaine adrenalin. An inverse bevel incision was used to get tissue from the underside of the papilla. The gingival samples included part of the pocket epithelium, connective tissue, and granulation tissue. After washing the samples in sterile 0.15 M saline solution, they were fixed in 10% formalin solution and sent to the Department of Pathology, Baskent University. Analyses were performed on coded samples by one of the authors (NB), who was masked with regard to the subjects’ diagnoses until all analyses were finished.

After the routine paraffin tissue procedures described above, tissues were embedded in paraffin blocks. Then, sections (4 to 5 μm thick) were obtained from the paraffin blocks, and serial sections were

† Carl Martin, Solingen, Germany
immunostained with an AEC (3-amino-9-ethylcarbazole) substrate system biotin-streptavidin complex system.\(^\text{§}\) AEC is a widely used chromogen for immunohistochemical staining. Deparaffinization and rehydration of the sections were followed by blocking endogenous peroxidase activity achieved by incubating the sections in 3% \(\text{H}_2\text{O}_2\) for 30 minutes at room temperature. After rinsing with distilled water, the sections were treated in a microwave oven with 10 mM citrate buffer (pH 6.0) at 500\(^\circ\)C for 5 minutes, at 600\(^\circ\)C for 4 minutes, and at 700\(^\circ\)C for 3 minutes for antigen retrieval. The slides were left to cool at room temperature for 50 minutes. After rinsing with phosphate buffered saline (PBS) for 2 to 3 minutes, the slides were kept in 3% \(\text{H}_2\text{O}_2\) for 20 minutes, and then rinsed with PBS for 5 minutes. Non-specific binding was reduced with protein-blocking serum for 20 minutes. Sections were incubated with iNOS primary polyclonal antibody\(^\text{i}\) at room temperature overnight. After rinsing with PBS for 2 to 3 minutes, sections were incubated with biotinylated goat antipolyvalent\(^\text{¶}\) for 20 minutes. The sections were washed with PBS for 2 to 3 minutes and treated with streptavidin peroxidase\(^\text{#}\) for 20 minutes, then washed again with PBS for 2 to 3 minutes. To visualize antibody binding, a biotin-streptavidin complex system was used, which was washed with distilled water, and the sections were counterstained with Mayer’s hematoxylin.

The morphologic identification of inflammatory cells was carried out in immunohistochemical slides. The latter were examined for positive staining by light microscopy and sections graded according to the modified scale system\(^\text{40,57-60}\) shown in Table 1. Because AEC was used as a color reagent, orange-brown staining of cytoplasm was considered positive for iNOS activity in inflammatory cells.

### Statistical Analyses

Statistical analyses were performed with a software program.** Normality was tested with the Shapiro-Wilk test. Because all clinical and histopathologic parameters were distributed normally, parametric tests were used to compare differences among the groups. The \(\chi^2\) test was used to compare gender distributions among the groups. One-way analysis of variance was used to compare the differences in mean age among the groups. Because there was a difference among groups with regard to the mean age, analysis of covariance was used to adjust for the differences.

Because inflammation intensity, iNOS expression, and the density of iNOS\(^\text{+}\) inflammatory cells were not distributed normally, the Kruskal-Wallis test was used to compare differences among the groups. The Spearman rho rank correlation coefficient was used to analyze the relationship among inflammation intensity, iNOS expression intensity, and the density of iNOS\(^\text{+}\) inflammatory cells. \(P\) values <0.05 were considered statistically significant.

### RESULTS

Patient characteristics and laboratory markers are shown in Table 2.

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**Table 1. Parameters Evaluated in Sections From Gingival Biopsy Samples**

<table>
<thead>
<tr>
<th>Inflammation Intensity</th>
<th>Area of iNOS(^\text{+}) Inflammatory Cells</th>
<th>iNOS Expression Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Minimal</td>
<td>&lt;20%</td>
<td>Weak</td>
</tr>
<tr>
<td>Mild</td>
<td>20% to 50%</td>
<td>Strong</td>
</tr>
<tr>
<td>Moderate</td>
<td>&gt;50%</td>
<td>3</td>
</tr>
<tr>
<td>Diffuse</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 2. Laboratory Markers of Subjects**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Fasting Plasma Glucose (mg/dl; mean ± SD)</th>
<th>HbA1c (%; mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM + CP</td>
<td>20</td>
<td>148.3 ± 41.1(^\text{*†‡})</td>
<td>7.5 ± 1.1(^\text{‡})</td>
</tr>
<tr>
<td>CP</td>
<td>20</td>
<td>87.2 ± 7.9</td>
<td>4.9 ± 0.7</td>
</tr>
<tr>
<td>DM</td>
<td>20</td>
<td>136.2 ± 34.5(^\text{‡†})</td>
<td>6.9 ± 1.2(^\text{‡†})</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>87.8 ± 7.3</td>
<td>5.0 ± 0.6</td>
</tr>
</tbody>
</table>

\(^*\) Significantly higher than the control group (\(P<0.01\)).

\(^†\) Significantly higher than the DM group (\(P<0.05\)).

\(^‡\) Significantly higher than the CP group (\(P<0.001\)).

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\(^\text{§}\) AEC + substrate chromogene, K3469, ready-to-use, DAKO, Glostrup, Denmark.

\(^\text{i}\) Rabbit polyclonal antibody RB-1605-R7, ready-to-use, Lab Vision, Freemont, CA.

\(^\text{¶}\) TP-060-HL, Thermo Scientific, Lab Vision.

\(^\text{#}\) TS-060-HR, ready-to-use, Thermo Scientific, Lab Vision.

\(^\text{**}\) SPSS for Windows, version 11.5, SSPS, Chicago, IL.
Clinical Analyses

The mean values for the periodontal measurements are shown in Table 3. PI, GI, PD, BOP, and CAL were statistically significantly higher in patients with DM and CP compared to patients with DM (P < 0.001); PI, GI, and PD were significantly higher in patients with DM and CP compared to controls (P < 0.001). There was no significant difference for any periodontal parameter between the patients with DM and CP and the patients with CP (P > 0.05). Although there were no significant differences for PI, PD, BOP, and CAL measurements between patients with DM and controls (P > 0.05), GI was significantly higher in patients with DM compared to controls (P < 0.05), and PI, GI, and PD were significantly higher in patients with CP compared to controls (P < 0.001), and PI, PD, BOP, and CAL were significantly higher in patients with CP compared to patients with DM (P < 0.001).

Immunohistochemical Analyses

Table 4 summarizes the results of the immunohistochemical analyses. Patients with DM and CP (Fig. 1A) had significantly greater numbers of inflammatory cells compared to controls (Fig. 1B) (P < 0.001) and patients with DM (Fig. 1C) (P < 0.01), but there was no difference between patients with DM and CP and patients with CP (P > 0.05). The density of iNOS-stained inflammatory cells was significantly higher in patients with DM and CP compared to controls (P < 0.001) and patients with DM (P < 0.05), and there was no difference between patients with DM and CP and patients with CP (P > 0.05).

Table 3.
Periodontal Parameters (mean ± SD) in the Study Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DM + CP Group</th>
<th>CP Group</th>
<th>DM Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
</tr>
<tr>
<td>PI</td>
<td>1.75 ± 0.38§</td>
<td>1.75 ± 0.34§</td>
<td>0.75 ± 0.32</td>
<td>0.66 ± 0.40</td>
</tr>
<tr>
<td>GI</td>
<td>1.54 ± 0.21§</td>
<td>1.70 ± 0.32§</td>
<td>0.51 ± 0.23¶</td>
<td>0.34 ± 0.29</td>
</tr>
<tr>
<td>PD (mm)</td>
<td>4.22 ± 0.47§</td>
<td>4.29 ± 0.61§</td>
<td>1.87 ± 0.23</td>
<td>1.74 ± 0.44</td>
</tr>
<tr>
<td>BOP (% of sites)</td>
<td>49.58 ± 9.04¶</td>
<td>61.3 ± 21.9§</td>
<td>4.35 ± 3.57</td>
<td>NA</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>6.05 ± 1.05II</td>
<td>5.93 ± 1.35II</td>
<td>2.57 ± 0.50</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not applicable.
§ Significantly higher than the control group (P < 0.001).
¶ Significantly higher than the DM group (P < 0.001).

Table 4.
Data for Inflammation Intensity, Density of iNOS+ Inflammatory Cells, and iNOS Expression Intensity for Each Group

<table>
<thead>
<tr>
<th></th>
<th>DM + CP Group (mean ± SD; median [range])</th>
<th>CP Group (mean ± SD; median [range])</th>
<th>DM Group (mean ± SD; median [range])</th>
<th>Control Group (mean ± SD; median [range])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation intensity</td>
<td>3.06 ± 1.10 3 (1 to 4)§§</td>
<td>2.85 ± 1.34 3 (0 to 4)§§</td>
<td>1.89 ± 1.40 1.5 (0 to 4)¶</td>
<td>0.70 ± 1.03 0 (0 to 3)</td>
</tr>
<tr>
<td>Incidence rate of iNOS+ inflammatory cells</td>
<td>2.41 ± 0.80 3 (1 to 3)§§</td>
<td>1.75 ± 1.02 2 (0 to 3)§§</td>
<td>1.56 ± 1.20 1.5 (0 to 3)¶</td>
<td>0.45 ± 0.61 0 (0 to 2)</td>
</tr>
<tr>
<td>iNOS expression intensity</td>
<td>1.71 ± 0.50 2 (1 to 2)§§</td>
<td>1.70 ± 0.66 2 (0 to 2)§§</td>
<td>1.17 ± 0.90 1 (0 to 2)¶</td>
<td>0.50 ± 0.69 0 (0 to 2)</td>
</tr>
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† Significantly higher than the DM group (P < 0.05).
§§ Significantly higher than the control group (P < 0.001).
¶¶ Significantly higher than the control group (P < 0.05).
All immunohistochemical parameters were significantly higher in patients with CP (\( P < 0.001 \)) and patients with DM (number of inflammatory cells and iNOS expression, \( P < 0.01 \); density of iNOS\(^+\)-stained inflammatory cells, \( P < 0.001 \)) compared to controls.

Table 5 shows the relationship among histopathologic parameters according to group. The intensity of inflammation was significantly correlated with the incidence rate of iNOS\(^+\)-stained inflammatory cells in patients with DM and CP.

In patients with CP, iNOS expression was significantly correlated with the intensity of inflammation and the incidence rate of iNOS\(^+\)-stained inflammatory cells.

The intensity of inflammation was highly correlated with the incidence rate of iNOS\(^+\) inflammatory cells and iNOS expression in patients with DM. Additionally, there was a highly significant correlation between the incidence rates of iNOS\(^+\) inflammatory cells and iNOS expression.

**DISCUSSION**

To our knowledge, this is the first report to study the expression of iNOS in the gingiva of patients with CP with and without DM. In the present study, there were slight, but not significant, differences between patients with CP and DM and patients with CP with regard to all periodontal parameters. Although all groups had a significantly large number of infiltrated inflammatory cells, iNOS\(^+\) cells, and higher iNOS expression in their gingival tissues compared to controls, there were slight, but not significant, differences between patients with DM and CP and patients with CP for all parameters. It is possible that the present periodontal condition was due to CP, and DM might not have had a significant impact on the clinical course of periodontitis in the current study. Accordingly, increased iNOS expression in patients with DM and CP did not seem to have an additional detrimental effect on ongoing periodontal inflammation in patients with DM than periodontitis alone.
Although several studies\textsuperscript{4-10,12-17,22-25,30,31,33,34} investigated the relationship between diabetes and periodontitis clinically, outcomes were often controversial. Diagnostic parameters and methodologies are not universally defined, which makes comparisons of the available evidence difficult. In subjects with diabetes, the onset and duration of the disease, the level of glycemic control, the type of treatment, and the presence of systemic complications vary.\textsuperscript{61}

In agreement with earlier findings,\textsuperscript{62-66} we did not find any significant difference for PI, GI, PD, BOP, and CAL between patients with CP and patients with CP and DM. Although Novak et al.\textsuperscript{67} also found no difference for PI, GI, PD, and BOP between patients with periodontitis and patients with diabetes and periodontitis, CAL was increased in patients with diabetes and periodontitis. However, Lucarini et al.\textsuperscript{68} and Correa et al.\textsuperscript{69} reported that patients with diabetes and periodontitis had significantly greater GI, PD, and BOP and increased CAL compared to patients with only periodontitis.

In another study,\textsuperscript{70} patients with periodontitis and inadequately controlled diabetes showed significantly higher PI and BOP compared to otherwise healthy patients with periodontitis. In the present study, PD and BOP were significantly higher in patients with CP and DM compared to patients with only DM. Although Takeda et al.\textsuperscript{25} also found significantly higher PD, contrary to our findings, they reported no difference for BOP. We found no significant differences for PI and PD,\textsuperscript{63,71} but a significant difference was found for GI between the group with DM and the control group. This may explain the increased levels of infiltrated inflammatory cells in

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<th>Table 5. Correlation Among Immunohistochemical Parameters for Each Group</th>
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<tr>
<td><strong>Group</strong></td>
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<td>DM + CP group</td>
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Boldface type indicates a statistically significant difference.
* Correlation is significant at the 0.05 level (two-tailed).
† Correlation is significant at the 0.01 level (two-tailed).
patients with DM compared to healthy controls in the present study. Recently, Lorentz et al.72 evaluated the progression of periodontitis among individuals attending periodontal maintenance programs; diabetes was not associated with periodontitis progression. Additionally, subjects with controlled diabetes do not show an increase in the extent and severity of periodontitis,61 which is concordant with our findings.

HbA1c is used routinely to monitor the glycemic control of patients with diabetes. In the present study, the conclusion that HbA1c was significantly higher in patients with DM and CP compared to patients with CP agreed with the findings from a previous study,73 and there was no difference in HbA1c between patients with DM and with or without periodontitis, as previously reported.25,74 In a study by Lim et al.,75 PD and BOP were evaluated in patients with diabetes; there was a significant positive correlation between PD/BOP and HbA1c. There are conflicting results regarding the relationship between glycemic control and periodontal status. HbA1c levels were decreased with periodontal treatment combined with31,34,76,77 and without33 antimicrobial therapy in patients with diabetes; however, contrary to these reports, the decrease did not reach significance in the studies by Promsudthi et al.17 and O’Connell et al.78 Very recently, it was reported that there was a slight, but not statistically significant, difference between patients with periodontitis and systemically and periodontally healthy individuals.79,80 After an adjustment for known risk factors for type 2 diabetes, periodontal disease severity was found to be related to HbA1c in subjects without diabetes.80 In the present cross-sectional study, periodontitis did not have a significant impact on HbA1c in patients with diabetes. The reason for this might be that all patients with diabetes had well-controlled disease. It is clear that HbA1c is not related to periodontal status in patients with controlled diabetes.

It is well known that NO plays an important role in the pathogenesis of chronic inflammation.81 Components of the host immune response that are not specific to given microbial pathogens, but that occur in response to infection, are likely to contribute to the pathogenesis of CP.82 This study is in agreement with earlier reports40-42,44,45 of iNOS expression in periodontitis. Infiltrated inflammatory cell intensity, iNOS positivity, and iNOS expression were significantly higher in patients with CP and were also higher in patients with diabetes compared to controls. These parameters were slightly, but not statistically significantly, higher in patients with DM and CP compared to patients with CP alone, and they were significantly higher in patients with DM and CP compared to patients with DM alone.

Several mechanisms have been proposed to explain the greater incidence and severity of periodontal disease in patients with diabetes. These include polymorphonuclear leukocyte changes,83 deregulated cytokine dysfunction, vascular changes, altered collagen and glycosaminoglycan synthesis, and the formation of AGEs.21,24 Patients without diabetes and periodontitis also showed iNOS expression and iNOS positivity in the present study.

iNOS is not usually expressed in non-inflamed healthy tissue,84 and elevated NO production is a reflection of an immune-activated state in which inflammatory cytokines and other mediators have upregulated iNOS.85 In the present study, despite no obvious clinical signs of inflammation, some inflammatory cells were present in the connective tissue of the healthy gingiva. Because the control subjects were free of immune deficiencies and inflammation, it is conceivable that persistent iNOS activity is responsible for the ongoing control of potentially infectious microorganisms in these tissues, suggesting a homeostatic function for iNOS.39

In the present study, inflammatory cell infiltration was significantly higher in patients with DM than in healthy individuals. Diabetes contributes to a hyperinflammatory state through the production of AGEs of proteins, which trigger monocyte/macrophage and cytokine production through interactions with receptors of AGE. This higher inflammatory state sets the stage for increased levels of periodontal disease triggered by oral pathogens.86 It was proposed that periodontal tissues are primed by a hyperinflammatory state and exhibit an exaggerated response to infecting organisms. The interaction of macrophages with AGEs was shown to stimulate increased secretion of proinflammatory mediators, such as TNF-α, IL-1 and -6, and prostaglandin E2.87-89 Periodontal tissue cells stimulated by periodontal disease–associated bacteria secrete proinflammatory cytokines, such as IL-1α, -1β, -6, and -8 and TNF-α, which play key roles in the pathogenesis of periodontal diseases; IL-1 and TNF-α are considered major mediators of periodontal inflammation.90-92 Bacteria also stimulate macrophages to generate NO,93,94 and iNOS expression can be induced by proinflammatory cytokines as well.38,93 The modulation of superoxide levels by NO influences phagocytic functions of neutrophils and macrophages, NO may be an important mediator of bone resorption, and iNOS influences osteoclast and osteocyte function in bone modeling.95,96

To the best of our knowledge, this study was the first to investigate the association among iNOS, type 2 diabetes mellitus, and periodontitis. Clinically, our results showed increased GI, PD, and BOP in patients with CP compared to patients with CP and DM. However, in the immunohistochemical examination,
infiltrated inflammatory cell intensity, iNOS positivity, and iNOS expression were higher in patients with DM and CP compared to patients with CP alone. In patients with DM and CP, although only inflammation intensity and the incidence rate of iNOS+ inflammatory cells were correlated, all parameters correlated with each other in patients with DM and in healthy individuals. In patients with CP, inflammation intensity was correlated with iNOS expression intensity, and the incidence rate of iNOS+ inflammatory cells was correlated with iNOS expression intensity. Therefore, it is possible to explain the increased expression of iNOS by increased inflammatory cells and iNOS positivity in healthy individuals and patients with DM but not in patients with DM and CP and patients with CP.

CONCLUSIONS
This article demonstrates that inflammation and iNOS expression are more prominent in the gingiva of patients with DM and CP. iNOS expression does not seem to be a predictor, but is a contributor, to the course of controlled diabetes mellitus presenting with periodontal inflammation. It is possible to speculate that diabetes and periodontal inflammation have a synergistic effect on inflammation, iNOS positivity, and iNOS expression. However, this effect does not seem to cause more severe periodontal tissue destruction than that caused by periodontitis alone.

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REFERENCES
25. Takeda M, Ojima M, Yoshioka H, et al. Relationship of serum advanced glycation end products with


47. Graier WF, Simecek S, Kukovetz WR, Kostner GM. High D-glucose-induced changes in endothelial Ca2+/EDRF signaling are due to generation of superoxide anions. *Diabetes* 1996;45:1386-1395.


95. van’t Hof RJ, Ralston SH. Nitric oxide and bone. *Immunology* 2001;103:255-261.


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