Endodontic Infection and Endothelial Dysfunction Are Associated with Different Mechanisms in Men and Women

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Abstract

Introduction: To investigate the potential link between apical periodontitis (AP) and cardiovascular (CV) function, inflammation markers, endothelial flow reserve (EFR), and levels of asymmetrical dimethylarginine (ADMA), the endogenous inhibitor of nitric oxide synthase (NOS), were measured in young adults with AP aged 20–40 years of both sexes. **Methods:** Forty men and 41 women (31 ± 5.71 years) free from periodontal disease, CV disease, and traditional CV risk factors were enrolled in the study. Twenty men and 21 women had AP; 40 healthy individuals matched for age, sex, and physical characteristics were also recruited as controls. All subjects underwent dental and complete physical examination, electrocardiography, conventional and tissue Doppler imaging echocardiography, and measurement of EFR. Interleukin (IL)-2, tumor necrosis factor alpha, reactive oxygen species (ROS), and ADMA were also assessed. Data were analyzed using the 2-tailed Student *t* test, the Pearson *t* test (or the Spearman *t* test for nonparametric variables), and multivariate linear regression analysis. **Results:** Echocardiography excluded any morphologic and functional cardiac alteration in all the subjects studied. Patients with AP of both sexes showed a significant reduction in EFR ($P < .05$) and a significant increase in IL-2 (men: $P < .01$, women: $P < .05$), whereas ROS were increased significantly only in women ($P < .05$). ADMA levels were unchanged in women with AP, but they were significantly increased in men ($P < .05$). A significant direct correlation between ADMA and IL-2 ($r = 0.67$, $P < .001$) and an inverse correlation between ADMA and EFR ($r = -0.42$, $P < .05$) in men and a significant inverse correlation between ROS and EFR ($r = -0.71$, $P < .01$) in female patients were observed. **Conclusions:** The presence of chronic inflammation in young adults with AP may cause early endothelial dysfunction documented by the reduced EFR. AP in men may influence the metabolism of NOS, whereas in women it appears to implicate a more direct detrimental mechanism. This difference is sex dependent and may be attributable to the protective action of estrogen in women. *(J Endod 2015;41:594–600)*

Key Words

Apical periodontitis, cardiovascular disease, markers of inflammation

Chronic inflammation plays a crucial role in the genesis of atherosclerosis and at the same time promotes acute cardiovascular (CV) events (1). Apical periodontitis (AP) is itself an inflammatory disease, most often chronic, of the periapical tissues in response to a polymicrobial infection of pulpal origin (2–4). To date, the relevant issue of a causal relationship between AP and the initial damage to the CV system has not been extensively investigated (5).

The equilibrium in the circulatory system is maintained by a normal functional endothelium able to inhibit platelet aggregation, monocyte adhesion, and vascular smooth muscle cell proliferation (6). Therefore, the relationship between a state of low-grade chronic inflammation, such as that which accompanies AP and CV distress, may be ascertained throughout early signs of vasomotion dysfunction.

We recently showed a reduced endothelial flow reserve (EFR) in young adult males with AP free of CV risk factors or CV disease (CVD) as assessed by echocardiography and tissue Doppler imaging (TDI) (5). EFR, a reproducible index of endothelial-dependent vasodilation, is indicative of the endothelial function and is measured at the level of the distal extremity in the upper limbs by means of peripheral arterial tonometry, a non–"operator-dependent" method (7). The arterial impairment in young adult males was associated with increased levels of proinflammatory cytokines (5). These substances, besides being sensitive systemic markers of tissue damage, have been shown to be predictive of future adverse cardiac events among healthy men (8). In these patients, higher concentrations of asymmetric dimethylarginine (ADMA), the endogenous inhibitor of the enzyme nitric oxide synthase (NOS), were also identified (5). Elevated plasma levels of ADMA are considered to be responsible for endothelial dysfunction (9, 10) and play a leading role as biological markers of atherogenesis (11–14). Based on this premise, the significant increase in plasma concentrations of ADMA observed by us in young male patients with AP and its inverse correlation with reduced EFR widely documented the presence of early endothelial impairment and should be considered as a predictor for the future development of CVD.

In that first study on AP and CV risk, the observation was limited to the male sex, assuming that the known protective activity of estrogen in young women could hide an aggressive inflammatory action on the CV system. The purpose of this study was to further investigate the association between endothelial dysfunction and chronic AP in adult patients of both sexes.

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Materials and Methods

Study Design and Patient Population

The present study is an observational cross-sectional trial conducted on young men and women observed at the Diagnostic Section of the Dental Clinic at the University of Cagliari, Cagliari, Italy. Subjects were divided into 4 groups: male and female patients affected by AP and healthy controls of both sexes. The study protocol was approved by the Institutional Ethics Committee (Azienda Ospedaliero Universitaria, University of Cagliari). Written informed consent was obtained from all subjects.

Inclusion criteria were as follows:

1. 20–40 years of age
2. The presence of AP as assessed both clinically and radiographically in at least 1 tooth (2) (teeth with apical symptoms but without radiographic appearance of AP were not included)
3. The presence of at least 25 teeth
4. Echocardiographic left ventricle ejection fraction (LVEF) value $\geq$55%
5. Hepatic and renal function within normal limits (bilirubin $\leq$1.5 mg/dL, creatinine $\leq$2.0 mg/dL)

Exclusion criteria were as follows:

1. The presence of localized or diffuse periodontal disease
2. The presence of nonendodontic lesions in the maxilla or the mandible
3. The presence of CV risk factors (arterial hypertension, dyslipidemia, diabetes mellitus, obesity, and present or past history of smoking)
4. Left ventricle (LV) hypertrophy at echocardiography
5. Previous and/or current CVD or cerebrovascular disease
6. The presence of chronic inflammatory conditions in other regions involving systemic health
7. For women, the use of anticonceptive hormones

Twenty-one female and 20 male white patients who consecutively registered for a dental checkup at the School of Dentistry and fulfilled the previously mentioned criteria were enrolled. Additional female and male patients (n = 20/group) who were free from clinical and radiographic evidence of AP and met the inclusion and exclusion criteria of the study were included as the respective control groups of women and men with AP. Patients in these control groups had similar demographic data (race, sex, and age distribution) to the experimental group. All individuals aged 31 $\pm$ 5.71 years underwent a complete CV assessment including medical history, physical examination, blood pressure measurement, 12-lead electrocardiogram, and conventional and TDI echocardiographic analysis. In addition, blood samples were collected for the assessment of circulating levels of interleukin (IL)-2, tumor necrosis factor alpha, reactive oxygen species (ROS), and ADMA.

Dental Examination

A complete dental examination was performed on each patient in both groups. A panoramic radiograph was obtained and examined for each patient and used as initial screening. The panoramic radiograph was followed by selected periapical radiographs taken on all teeth suspected of presenting AP, all teeth showing the presence of root canal treatments, and all teeth showing the presence of restorations (including prosthetic restorations) with or without previous endodontic treatment.

Periapical radiographs were taken with a radiographic unit (Castellini; Cefla, Imola, Italy) using a long cone paralleling technique (70 Kv, 10 Ma, film-focus distance = 28 cm) and Ultra Speed film (Eastman Kodak, Rochester, NY). Radiographs were examined in a darkened room by an endodontist with 20 years of practice using an illuminated viewer box with 3.5$\times$ magnification to assess the presence of a periapical lesion involving at least 1 root of a given tooth. Intraoral examination was then completed. Using both radiographic and intraoral evaluations, the following parameters were recorded:

1. The number of teeth present
2. The number and location of restored teeth
3. The number of endodontically treated teeth
4. The number and location of teeth affected by carious processes
5. Soft tissue assessment (the presence and location of swelling/sinus tracts)
6. Periodontal probing
7. Sensitivity tests were performed in all teeth using both cold and electric pulp tester
8. The number and location of teeth with AP
9. Radiographic appearance of the mandibular and maxillary bones (to exclude the presence of nonendodontic lesions or other possible findings; ie, impacted teeth)

Conventional Echocardiography and TDI

All study participants were instructed on the medical environment and instrumentation before testing. Echocardiographic images were recorded using a system equipped with TDI imaging and raw data acquisition (Toshiba Aplio; Toshiba Corp, Tochigi, Japan). LVEF was obtained according to Simpson's rule and considered abnormal when <55%. By means of pulsed wave Doppler, the early and late diastolic peak velocities and E deceleration time were measured, and the early/late ratio was subsequently derived. Longitudinal function was assessed using pulsed TDI at the mitral annulus; peak velocities in systole, early, and late diastole were measured. LV longitudinal function was evaluated off-line from raw data (TDI-Q, Toshiba Corp); longitudinal strain (Σ) and strain rate (average from basal and midsegments of 4-chamber and 2-chamber views) were also quantified based on TDI. All the examinations on each patient were performed by a single experienced echocardiographer. Reproducibility of TDI parameters in our laboratory has been previously documented (15).

Inflammatory and Oxidative Stress Markers

In all subjects, a blood sample was obtained by venipuncture of the antecubital vein after overnight fasting. Levels of IL-2 and TNF-α were determined by enzyme-linked immunosorbent assay (Immunotech, Marseille, France) and expressed in pg/mL. ROS blood levels were determined on fresh heparinized blood samples using the free oxygen radicals test (FORT). Results are expressed as FORT units (U) where 1 FORT U corresponds to 0.26 mg/L H_2O_2.

ADMA

Plasma ADMA was analyzed by high-pressure liquid chromatography (HPLC) coupled with laser-induced fluorescence detection. Blood samples collected in serum tubes were centrifuged and extracted by solid phase extraction (SPE) at room temperature. After extraction, ADMA was quantified by the chromatographic method.

Endothelial Function

EFR was measured at the level of the distal extremity in the upper limbs by means of peripheral arterial tonometry. The ENDO-PAT 2000 model (Itamar Medical, Columbia, SC), an apparatus that measures changes in vascular tone influenced by the endothelium in the fingers