Association among Oral Health, Apical Periodontitis, CD14 Polymorphisms, and Coronary Heart Disease in Middle-aged Adults

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Abstract

Introduction: There is evidence to suggest that an association exists between oral infections and coronary heart disease (CHD). Subjects presenting lesions of endodontic origin (LEOs) or pulpal inflammation had an increased risk of developing CHD. However, findings concerning systemic manifestations of apical periodontitis (AP) remain controversial. An association between CD14 gene polymorphisms and atherosclerosis-associated diseases has been shown, but there are no data regarding an association between CD14 polymorphism and AP. This study evaluated associations between clinical oral health status, CD14 polymorphisms, and CHD. **Methods:** A case-controlled clinical trial was designed to compare middle-aged adults with acute myocardial infarction or unstable angina (n = 51) within 12 months of the acute event defined as first manifestation with healthy controls (n = 49). Participants were matched for age, sex, and socioeconomic status. Indicators of oral disease and compliance were evaluated. CD14 polymorphisms were analyzed by restriction fragment length polymorphism–polymerase chain reaction. **Results:** CHD subjects had a higher prevalence of oral diseases and lower compliance to oral preventive strategies than healthy controls. Multivariate analysis showed a positive association between missing teeth (odds ratio [OR] = 1.37; 95% confidence interval [CI], 1.02–1.85), the number of LEOs (OR = 4.37; 95% CI, 1.69–11.28), chronic periodontitis (OR = 5.87; 95% CI, 1.17–29.4), and CHD. No statistically significant association emerged between the CD14 C(−260)T and the CD14 C(−159)T polymorphism, endodontic or periodontal disease, and CHD. **Conclusions:** Chronic oral diseases may increase the risk of CHD and may be an unconventional risk factor for CHD. *(J Endod 2012;38:1570–1577)*

Key Words

Cardiovascular disease, coronary heart disease, oral disease, chronic periodontitis, apical periodontitis, CD14, polymorphisms

Cardiovascular diseases (CVDs) are the leading cause of death and hospitalization for both sexes in nearly all European countries. Among CVDs, ischemic heart disease alone is the single most common cause of death in Europe (1). However, it is largely preventable through the identification of high-risk individuals. Conventional risk factors for atherosclerosis and coronary heart disease (CHD) such as hypertension, diabetes, sex, socioeconomic status, smoking, obesity, high low-density lipoprotein (LDL) serum levels, and genetic disposition have been clearly established (2).

In young patients, other risk factors such as smoking and family history (3) appear to play significant roles in heart disease. Recently, unconventional factors such as the presence of chronic inflammatory diseases have also been considered as correlates of heart disease. Circulating markers of activated inflammation and hemostatic factors are closely associated with the development of fatal and nonfatal myocardial infarction (4). Thus, chronic inflammatory processes may be considered predictors for atherosclerosis. Chronic oral infections in particular have been associated with CHD (5–7).

Chronic periodontitis is associated with atherosclerosis and an increased prevalence and incidence of ischemic heart disease independent of conventional risk factors (8). Periodontal infection may influence the development of CHD by hematogenous exposure to gram-positive and gram-negative bacteria through a mechanism of molecular mimicry. The binding of lipopolysaccharide (LPS) with CD14 receptors present on the endothelial membrane is thought to cause the release of proinflammatory factors and the exposure of adhesins involved in the processes of atherogenesis and thrombogenesis (9). The presence of *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Aggregatibacter actinomycetemcomitans* DNA has also been shown in atheromas (6). Chronic periodontitis induces an increase in C-reactive protein (CRP) and antiperiodontal pathogen IgA antibodies (10). Increased CRP has been associated with an increased risk of CVD in healthy individuals (11). Recent studies have detected elevated CRP levels, both in patients with coronary atherosclerosis and in those with periodontal disease (12), with the biggest increase found in patients suffering from both diseases. Chronic periodontitis has also been associated with increased levels of total cholesterol, triglycerides, and LDL and with reduced levels of high-density lipoproteins (HDLs). Furthermore, after periodontal treatment, an increased serum concentration of HDL

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cholesterol has been found, with a consequent increase in the HDL/LDL ratio and reduced serum levels of CRP (13).

Apical periodontitis (AP), as an immune response to chronic bacterial contamination of the endodontic and periradicular spaces, presents significant similarities with the inflammatory response involved in chronic periodontitis (14). Endodontic pathogens, which tend to be the same as those involved in periodontal infections, may reach the atherosclerotic plaques through a mechanism of metastatic infection or may act through the previously mentioned mechanism of molecular mimicry. An increase in proinflammatory cytokines has been reported in the pulp, periapical tissues, and serum of patients with pulpitis and apical periodontitis (15, 16). In particular, interleukin 1, interleukin 6, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor α promote the activation of neutrophilic granulocytes, monocytes/macrophages, the alternative complement pathway, and hemostasis. These cytokines also increase the binding strength of LDL to the endothelium and smooth vessel musculature. Furthermore, they increase expression of the gene for the vascular receptor of LDL favoring atherosclerosis (14). Periradicular inflammatory lesions of endodontic origin (LEOs) have also been associated with increased levels of serum CRP in the same manner observed in periodontal disease (17). Findings concerning systemic manifestations of periradicular inflammatory processes remain controversial. However, some studies have shown an increased risk of developing ischemic heart disease in subjects presenting with periradicular LEOs (18) or pulpal inflammation using root canal treatment (RCT) as a surrogate parameter (15, 19).

Genetic disposition to CHD can be assessed by examining any family history of early CHD, the genetic determinants of phenotypes involved in the pathophysiology of CHD, the analysis of gene-environment interaction, and the role of genetic polymorphisms (20). A large number of candidate genes have already been investigated in relation to CHD traits and the risk of CHD itself, such as the C(−260)T single-nucleotide polymorphism in the promoter region of the CD14 receptor gene and the C(−159)T in the CD14 gene. The innate immune system is the first line of defense against invading microorganisms. It is activated through the recognition of pathogen-associated molecules such as bacterial cell wall components. CD14, a 55-kd membrane glycoprotein expressed predominantly on the surface of monocytes/macrophages and neutrophils, plays a crucial role in the recognition of several microbial products such as LPS and peptidoglycan, which are major components of gram-negative and gram-positive bacterial surfaces, respectively (21). The LPS- or peptidoglycan-CD14 complex, together with other accessory proteins, interacts with the cell-surface Toll-like receptors. The activation of multiple signaling pathways leads to the up-regulation of inflammatory cytokines (22). CD14 is expressed on the cell surface via a glycosylphosphatidylinositol anchor but is also found free in plasma; it is referred to as soluble CD14 (sCD14). sCD14 mediates the LPS activation of CD14-negative cells including endothelial and epithelial cells (23). Recent studies have shown the role of the CD14 C(−260)T gene as a genetic marker of susceptibility for atherosclerosis; the effect of the T allele is more evident in later stages of disease (24). Furthermore, a higher frequency of the T allele appears to be correlated to an increased amount of sCD14 in patients. The TT genotype of CD14 C(−260)T and CD14 C(−159)T genes has been proposed as a risk factor of myocardial infarction (25). Any association between CD14 and chronic periodontitis remains controversial (26, 27), and no data exist about endodontic disease. The aim of this study was to evaluate the association between clinical oral health status and the presence of periradicular LEOs, CD14 polymorphisms, and CHD by comparing middle-aged adults affected by CHD with a healthy control group.

Materials and Methods

The study was authorized by the SG Battista University Hospital Ethics Committee and the Piedmont Regional Health System Review Board. All subjects gave informed written consent for participation in the study, which was approved by the institutional ethic committee and performed according to the principles of the last update of the Helsinki Declaration.

An observational case-controlled clinical trial was designed. Dentate subjects with a minimum of 5 teeth, as suggested in a previous study (28), and no RCT in the last 2 years, were enrolled according to the following inclusion criteria: below 55 years of age; no medical history of diabetes or systemic, oncologic, or immune system diseases; no immune-suppressive or cortisone drug treatment in progress; and normal weight (ie, body mass index [BMI] <25 kg/m^2). The CHD group comprised the first consecutive, informed, and cooperating patients with a diagnosis of acute myocardial infarction (STEMI) or unstable angina (UA/NSTEMI). Subjects were recruited within 12 months of the acute event defined as first manifestation without known conventional risk factors for coronary disease except for smoking and family history and examined at the Cardiology Department of San Giovanni Battista University Hospital, Turin, Italy, between October 2009 and June 2010.

Healthy controls were randomly enrolled from a general medical database from the same district as the CHD group using a computer-generated random numbers sequence and then matched for age (matching range ± 2 years), sex, and socioeconomic status with the CHD group. The presence of CVD was excluded in the control group before data collection through a cardiologic checkup and electrocardiogram.

In both groups, the medical history of cardiovascular disease among first-degree relatives (parents, siblings: aged below 55 years for male relatives and below 65 years for female relatives at the time of the ischemic episode) was evaluated. This independent risk factor, which is more significant in younger subjects than in the elderly, was included in the multivariate statistical analysis.

A structured patient history including demographic and socioeconomical status (ie, age, sex, occupation, and qualifications), medical and behavioral factors (ie, smoking and compliance with preventive strategies [frequency of dental appointments and professional oral hygiene (OH) sessions]), and previous dental history (ie, prior treatment and causes of tooth loss) was collected before dental examination. Smoking and family history were considered to be confounding factors of the association and were included in the statistical analysis.

The intraoral examination was performed using 3.5× Galilean loupes (Orascoptic, Middleton, WI) by the same previously calibrated examiner who was blinded to the participants' CHD status. For each patient, pulpal and periradicular status was assessed through vitality thermal and electric pulp tests (Diagnostic Unit; Sybron, Orange, CA), palpation, and percussion. Complete periodontal charting, including the full-mouth plaque score (FMPS), was recorded. A full-mouth radiographic examination was performed (Planmeca Intra, Helsinki, Finland) using Rinn XCP devices (Rinn Corp, Elgin, IL) and phosphor sensor imaging plates. The images were processed and archived by a dedicated scanner and software interface (OpTime; Soredex, Tuusula, Finland). Radiographs were analyzed by 3 clinical assistant professors at the endodontics department who were blinded to the CHD status of each subject.

Examiners' performances were calibrated on the evaluation criteria by means of a case series presentation until interexaminer reliability could be expected; concordance between examiners was analyzed through the Fleiss κ score ($\kappa > 0.70$). In cases of

nonunanimous opinion, the majority opinion was accepted. For each patient, the following indicators of dental disease were evaluated:

1. The number of missing teeth
2. Caries history (delayed missing filled teeth [DMFT]) and the number of untreated dental caries
3. Pulpal (ie, vital and nonvital) and periradicular status, the presence of LEOs and RCT teeth, and LEO-associated previous RCT (Teeth were classified as having LEOs by the loss of lamina dura and periodontal ligament enlargement of more than 2 mm as the largest diameter.)
4. Periodontal status according to the American Academy of Periodontology classification of periodontal diseases and conditions (29)

DNA Extraction and Analysis of Genetic Polymorphisms

The third finger tip of 1 hand of each subject was cleaned with antiseptic wipes and punctured with a sterile lancet. Blood samples were collected with a heparinized capillary, and DNA was extracted using the Extract-N-Amp blood polymerase chain reaction (PCR) kit from Sigma Chemical Co (St Louis, MO) containing all the reagents needed to rapidly extract and amplify human genomic DNA from whole blood. Briefly, 10 μL whole blood was mixed with 20 μL extraction solution, and the mixture was incubated at room temperature for 5 minutes. Neutralization solution (180 μL) was added, and PCR was performed. An aliquot of the neutralization extract was combined with the Extract-N-Amp Blood PCR Ready Mix (containing buffer, salts, dNTPs, Taq polymerase, and JumpStart Taq antibody) and user-provided PCR primers to amplify the target DNA.

Detection of Polymorphisms

PCR primers were synthesized by Sigma-Aldrich (Milan, Italy), and restriction enzymes were obtained from Promega (Madison, WI). Electrophoresis reagents were obtained from Bio-Rad Laboratories (Hercules, CA). The PCR–restriction fragment length polymorphism (PCR-RFLP) method was determined according to the procedure of Baldini et al (30) and Ito et al (31), respectively, as follows:

1. CD14 promoter −159 (C→T) polymorphism: primers: A: 5′-GTGCCAACAGATGAGGTTCAC-3′; and B: 5′-GCCTCGAGAGTTTATG TAATC-3′ (1 μmol/L); cycling: 96°C for 1 minutes, 38 cycles of 96°C for 40 seconds, 56°C for 40 seconds, 72°C for 40 seconds, and 72°C for 10 minutes. PCR products were digested for 1 hour at 37°C and inactivated at 65°C for 15 minutes with AvaII (2 U/10 μL reaction mixture). The restriction pattern was visualized by 0.2 μg/mL ethidium bromide staining after electrophoresis of the PCR-RFLP products (10% polyacrylamide gel, 150 V, 30 minutes). This procedure produced 2 fragments (353 bp and 144 bp) in subjects homozygous for allele T (−159 T) or 1 fragment of 497 bp in subjects homozygous for allele C (−159 C). All 3 fragments were present in heterozygous subjects.
2. CD14 promoter -260 (C→T) polymorphism: primers: A: 5′-CAAGG CACTGAGGATCATCC-3′ and B: 5′-CATGGTCGATAAGTCTTCCG-3′ (1 μmol/L); cycling: 85°C for 15 minutes, 94°C for 4.5 minutes, 42 cycles of 94°C for 30 seconds, 55°C for 1 minute, 72°C for 40 seconds, and 72°C for 7 minutes. PCR products were digested for 1 hour at 37°C with Hae III (2 U/10 μL reaction mixture).

The restriction pattern was visualized by 0.2 μg/mL ethidium bromide staining after electrophoresis of the PCR-RFLP products (10% polyacrylamide gel, 150 V, 30 minutes). This procedure produced 1 fragment of 418 bp in subjects homozygous for allele T (−590 T) or 2 fragments (263 and 155 bp) in subjects homozygous for allele C (−590 C). All 3 fragments were present in heterozygous subjects. To check the validity of the methods, 10 samples were genotyped twice with identical results.

Statistical Analysis

The Kolmogorov-Smirnov test for normality was used to check data distribution. The difference between groups was analyzed through inferential analysis. The Student *t* test was used for continuous normally distributed variables (FMPS and DMFT). The nonparametric Mann-Whitney *U* test was used for nonnormally distributed variables (ie, OH per day, number of missing teeth, untreated caries, LEOs, RCT teeth, and LEO-associated RCT teeth). The chi-square test was used for dichotomous variables (ie, family history, smoking, prevalence of chronic apical periodontitis, chronic periodontitis, combined endoperio diseases, and healthy subjects). The level of statistical significance was set at $P < .05$. A multivariate logistic regression model was used to analyze the effects of each variable on CHD risk. Estimates were shown as odds ratio (OR) and relative 95% confidence intervals (CIs) reciprocally adjusted for clinical factors, family history, and smoking. Subjects were classified in the adjustment setup of the regression analysis as smokers (current smokers + ex-smokers at any condition, duration of smoking habits, and quantity) and nonsmokers (never smoked). A descriptive analysis was used to evaluate the frequency of the CD14 polymorphisms of interest; the association with the risk of CHD was evaluated through a crude estimation of ORs and relative 95% CIs. All statistical analyses were performed using the SPSS for Windows 17.0 software package (SPSS Inc, Chicago, IL).

Results

Kappa values estimated to evaluate interexaminer reliability showed a high concordance between the raters (ie, 1 vs 2 [K = 0.73; 95% CI, 0.43–1.02], 1 vs 3 [K = 0.76; 95% CI, 0.48–1.04]; and 2 vs 3 [K = 0.83; 95% CI, 0.60–1.06]). Fifty-one patients were enrolled in the CHD group (40 men vs 11 women, mean age = 48 ± 5.7 years), and 49 subjects were enrolled in the control group (39 men vs 10 women, mean age = 47 ± 7.1 years). In the CHD group, 29.3% of patients had a family history of CHD compared with 7.1% in the control group. This difference was not statistically significant (Table 1). Enrollment rates were 80% and 65% in the CHD and control groups, respectively. The distribution of occupations and education in the 2 groups was descriptively compared and was relatively uniform with similar socioeconomic middle-class backgrounds.

Behavioral Factors: Smoking, Compliance, and Oral Health

Behavioral, compliance, and oral health indicators are summarized in Table 1. Smokers were significantly more prevalent in the CHD group at the time of the ischemic event compared with healthy controls (53.7% vs 10.7%, respectively). Of current smokers in the CHD group, 45.5% smoked fewer than 10 cigarettes per day, 27.3% smoked 10–20 cigarettes per day, and 27.2% smoked more than 20 cigarettes per day. Of the ex-smokers (22% of the group), 55% had stopped smoking within the last year, 22.5% had stopped smoking 1–5 years previously, and 22.5% had stopped more than 20 years previously. Current smokers and ex-smokers in the CHD group were exposed to tobacco abuse for an average period of 30 ± 6 years. Only 24.3% of the subjects in the CHD group had never smoked.

In the control group, one third of smokers reported smoking fewer than 10 cigarettes per day, and two thirds smoked 10–20 cigarettes per day. Of the ex-smokers (17.9%), 20% had stopped within the last 10 years, and 80% had stopped smoking more than 20 years

TABLE 1. Descriptive Statistics of Variables of Interest

	CHD patients	Healthy controls	*P* value
Family history (%)	29.3	7.1	.06
Smoking habits (%)	53.7	10.7	.001
OH and compliance			
FMPS (%)	73.2	58.5	.007
OH per day	1.6 (0.6) [2]	2.5 (0.7) [2]	<.001
Last visit DDS (m)	18.7 (15.2)	9.1 (9.2)	.001
Last visit RDH (m)	20.9 (18.8)	10.7 (12.6)	.01
Oral health status			
Missing teeth	5.7 (5.1) [4]	2.2 (2.6) [1.5]	.002
DMFT	15.6 (5.5)	12.8 (5.7)	.05
Number of caries	3 (2.28) [2]	2.2 (3.1) [1]	.21
Endo	85.3%	53.5%	.02
Number of LEOs	3.4 (2.7) [3]	1.1 (1.6) [1]	<.001
RCT teeth	3.5 (3.5) [3]	2.6 (2.6) [2]	.24
LEO*RCT	3 (2.8) [2]	1 (1.5) [1]	.002
Perio (%)	82.9	42.8	.005
Endoperio (%)	68.2	21.4	.001
Healthy (%)	0	25	.08

CHD, coronary heart disease; DDS, dentist; DMFT, delayed missing filled teeth; Endo, diagnosis of at least 1 LEO; Endoperio, diagnosis of both LEO and perio lesions; Healthy, rate of orally healthy subjects; LEO*RCT, lesions of endodontic origin associated with root canal treated teeth; OH, oral hygiene; Perio, prevalence of chronic periodontitis; RDH, dental hygienist.
Values are mean, (standard deviation), and [median].

previously. Current smokers and ex-smokers in the control group were exposed to tobacco abuse for an average period of 23 ± 8 years. However, 71.4% of subjects in the control group had never smoked.

Participation in an oral health preventive program through routine dental appointments and professional dental hygiene sessions was assessed along with the OH performance of subjects in both groups. The CHD group was less compliant with preventive strategies with significantly longer intervals between dental appointments and professional dental hygiene sessions compared with the control group. A comparison of daily OH frequency and FMPS suggested a less consistent and less efficacious OH performance in the CHD group compared with the control group.

Oral Health Status

Oral health status indicators and the statistical significance of differences between groups are summarized in Table 1. The number of missing teeth, considered as an indicator of previous irreversible oral diseases, was significantly higher in the CHD group compared with the control group (5.7 vs 2.2, respectively). DMFT values were significantly higher in the CHD group (Table 1). Mean DMFT values in the CHD group (15.6 ± 5.5) were also higher than the World Health Organization mean range (9–13.9) for subjects in the same age group/area. Mean DMFT values of the test group (12.8 ± 5.7) were instead in line with available World Health Organization epidemiologic data (http://www.who.int/oral_health/media/en/orh_report03_en.pdf). Furthermore, CHD subjects presented more untreated caries than the control group although the difference was not statistically significant.

The prevalence of AP was higher in the CHD group (85.3%) than the control group (53.5%). Subjects in the CHD group also had a higher mean number of LEOs per person compared with the control group (3.4 vs 1.1, respectively). Patients in the CHD group had received more frequent RCT than those in the control group (3.5 vs 2.6, respectively) although no significant differences existed between the groups. However, RCT in the CHD subjects appeared to have had a higher failure rate and were more frequently being associated with an LEO compared with the control group (85.7% vs 38.5%, respectively).

The prevalence of chronic periodontitis was significantly higher in the CHD group compared with the control group (82.9% vs 42.8%, respectively). Furthermore, the prevalence of a combined diagnosis of LEOs and lesions of periodontal origin was significantly higher in CHD subjects than in controls (68.2 vs 21.4%, respectively). No subject in the CHD group had total oral health; all showed at least 1 LEO or a diagnosis of chronic periodontitis. In comparison, 25% of subjects in the control group had complete oral health.

Multivariate analysis reciprocally adjusted for clinical variables (Table 2, column A) showed a significantly positive association between the number of missing teeth, the number of LEOs, the diagnosis of chronic periodontitis, and CHD. The number of caries also showed a positive but not statistically significant association. Other variables (ie, DMFT, the diagnosis of chronic apical periodontitis with the presence of at least 1 LEO, and the number of RCT teeth) did not show any positive association. Among oral health indicators, chronic periodontitis showed the strongest association with CHD (OR = 5.87). However, the amplitude of the 95% CI range indicates that the estimate is more uncertain than those of other variables. The association was still present after adjustment for family history but not after adjustment for smoking, possibly because of the impact of smoking and family history or the effect of stratification on the sample size. The association between the number of LEOs and the risk of CHD was still evident (Table 2, column

TABLE 2. Multivariable Logistic Regression Analysis of the Variables of Interest (ORs and relative 95% CIs)

	A			B			C			D		
		95% CI			95% CI			95% CI			95% CI	
Clinical factors	OR	Lower	Upper	OR	Lower	Upper	OR	Lower	Upper	OR	Lower	Upper
Missing teeth	**1.37**	**1.02**	**1.85**	**1.36**	**1.02**	**1.83**	1.35	0.95	1.91	1.35	0.94	1.92
DMFT	0.81	0.62	1.06	0.81	0.62	1.07	0.85	0.64	1.12	0.87	0.65	1.15
Caries (number)	1.28	0.95	1.73	1.30	0.96	1.77	1.18	0.81	1.74	1.21	0.81	1.81
Endo	0.89	0.13	6.28	0.86	0.12	6.34	0.71	0.16	17.98	1.40	0.12	16.04
LEO (number)	**4.37**	**1.69**	**11.28**	**4.09**	**1.59**	**10.54**	**4.79**	**1.79**	**12.81**	**4.45**	**1.66**	**11.89**
RCT teeth (number)	0.54	0.29	1.01	0.58	0.30	1.08	0.47	0.24	0.91	0.49	0.25	0.97
Perio	**5.87**	**1.17**	**29.4**	**5.05**	**0.99**	**25.85**	5.15	0.81	32.76	3.67	0.52	25.62
Family history				2.37	0.30	18.48				4.48	0.44	45.03
Smoking							**13.64**	**2.01**	**92.51**	**17.86**	**2.33**	**137.1**

DMFT, delayed missing filled teeth; Endo, diagnosis of at least 1 lesion of endodontic origin; LEO, lesions of endodontic origin; Perio, prevalence of chronic periodontitis; RCT, root canal treated.
Boldface type indicates statistical significance (*P* value <.05). Columns are as follows: (A) ORs reciprocally adjusted for clinical factors; (B) adjustment for clinical factors and family history; (C) adjustment for clinical factors and smoking; and (D) adjustment for clinical factors, smoking, and family history.

B) after adjusting for all confounding factors, showing a statistically significant stability of the estimates.

Polymorphism Analysis

Based on the data obtained in this study, the presence of the genotypes CC, CT, and TT in the analyzed white population were 18%, 42%, and 40%, respectively, for the CD14 polymorphism C(−159)T and 18%, 20%, and 62%, respectively, for allele CD14 C(−260)T. The frequencies of alleles C and T were 39% and 61% for the CD14 polymorphism C (−159), respectively. The frequencies of alleles C and T were 28% and 72% for CD14 polymorphism C(−260)T, respectively. The CD14 C(−159)T and CD14 C(−260)T polymorphisms were evaluated in the healthy control and CHD groups (Table 3). After adjusting for the presence of endodontic and periodontal disease, only the polymorphism of CD14 C(−159)T gene seemed to evidence a positive association with CHD. This was not statistically significant (OR = 3.13; 95% CI, 0.87–11.29). Furthermore, the effect of the T allele of the same polymorphic gene seemed to evidence an association with an increased risk of CHD (OR = 2.02; 95% CI, 0.82–4.96). This was not statistically significant. No statistically significant associations were found between the studied CD14 polymorphisms and endodontic and periodontal disease.

Discussion

CHD is not common in patients younger than 55 years old. However, for those who suffer from CHD at a young age, the disease can impose a significant morbidity, psychologic effect, and financial constraint (32). In this study, the test group consisted of an infrequent population of CHD patients aged below 55 years and recruited within 12 months of the first acute event with the typical risk profile for CHD of this age group. It may be assumed that smoking, family history, and unconventional risk factors including genetic factors and chronic inflammatory status rather than major risk factors that affect older people could have been disease determinants in this study population.

In this study, we only recruited patients who survived the CHD event. Those with fatal events could have not been included, and this may represent a potential inclusion bias. Coronary interventions, fibrinolytic agents, antithrombotic therapy, and secondary prevention have reduced the overall 1-month mortality of STEMI to 4%–6% of patients treated in the hospital. The timelines of care rather than the type of reperfusion (coronary interventions vs antithrombotic therapy) are the most important determinant of a favorable outcome for patients affected by STEMI (33). Hospital mortality is higher in patients with STEMI than among those with UA/NSTEMI (7% vs 3%–5%, respectively). During the enrollment period, all patients treated for STEMI in the coronary unit of Turin Molinette Hospital, Turin, Italy, were subjected to early reperfusion (percutaneous coronary intervention) within 90 minutes from the onset of symptoms, and no fatal events occurred. The same favorable outcome occurred for patients with UA/NSTEMI during the enrollment period. However, no clinical oral health data are available concerning CHD subjects eligible for this study who eventually died out of the hospital.

Patients affected by CHD had a significantly higher prevalence of chronic oral diseases and poorer compliance to oral health behaviors compared with the control group although their socioeconomic background was similar. A strong age-dependent association between poor oral health and sudden cardiac death has been shown by a previous study (34). Dental pathological lesions were prevalent among victims who suddenly died out of the hospital. Among men below the age of 50 years old, poor oral health seemed to be a significant risk factor for prehospital sudden cardiac death (34). A significant age-dependent association between the incidence of LEOs and the time to CHD diagnosis was also found by Caplan et al (18) among subjects below 40 years of age.

As expected, the 2 major risk factors for CHD in the adult population below 55 years of age, (ie, smoking and family history) were more prevalent in the CHD group. Smoking was highly prevalent in the CHD group (53.7%) even in comparison with Italian public health data (updated until May 2011: http://www.iss.it/fumo/index.php?lang=2). National monitoring for smoking, alcohol intake, and drug usage reports a smoking frequency of 22.7% among adults with a peak of 28.3% in the 25- to 44-year age range. Smokers made up just 10.7% of the control group in this study, confirming smoking as a predominant risk factor for CHD. Smoking increases the risk of endothelial injury to the peripheral vascular system and the development of a chronic inflammatory state, both of which are risk factors for CHD and the incidence of RCT. Compared with those who had never smoked, current cigarette smokers were 1.7 times more likely to have RCT (35). Current smokers and ex-smokers (at any condition, duration of smoking habit, and quantity) were classified together in the regression analysis as smokers and compared with nonsmokers (never smoked). As reported in the 2012 European Society of Cardiology Guidelines (http://www.escardio.org/guidelines-surveys/esc-guidelines/Pages/cvd-prevention.aspx) and the 2010 US Report of the Surgeon General (http://www.surgeongeneral.gov/library/reports/tobaccosmoke/index.html), there is a significant decrease in mortality and morbidity after only 6 months of smoking cessation. However, the cardiovascular risk significantly decreases to the lowest level after only 10–15 years of smoking cessation and never to the never smokers' value. Because it is not possible to quantify the impact of different modalities of cessation (ie, how many years since cessation and how many years of smoking a certain dose per day) on the deleterious effect of smoking, we considered ex-smokers together with smokers just as "exposed subjects" in the adjustment of the regression analysis. The previously mentioned reports state that there is a clear, nonlinear dose-response relationship for the risks associated with smoking. A sharp increase at a low level of exposure was reported together with a shallower dose-response relationship as the number of cigarettes increased. However, a significant risk appears to be more correlated with continuative smoking itself than differences in the number of cigarettes per day or the type of cigarettes. Also, noninhaled smoke is associated with an increased risk of CHD.

Only white subjects presented for enrollment in the study. Race itself is not yet considered a cardiovascular risk factor, so this should not

TABLE 3. Distribution of Genotype (%) and Allele Frequencies (%) of −159 (C→T) and −260 (C→T) Polymorphisms of the CD14 Gene

Polymorphism of CD14 gene	CHD patients (n = 51) %	Healthy subjects (n = 49) %
−159 (C→T) CD14		
Genotype		
CC	13.6	25
CT	45.4	37.5
TT	41	37.5
Allele		
C	36	44
T	64	56
−260 (C→T) CD14		
Genotype		
CC	20	15.7
CT	30	10.6
TT	50	73.7
Allele		
C	35	21
T	65	79

be considered a limit in the external validity of the study (http://www.escardio.org/guidelines-surveys/esc-guidelines/Pages/cvd-prevention.aspx). Genetic information is mainly represented by family history evaluation, the study of genetic determinants of phenotypes involved in the pathophysiology of CHD, gene-environment interactions, and genetic polymorphisms.

In this study, we included only subjects of normal weight corresponding to a BMI <25 kg/m^2. Overweight was defined by a BMI ranging between 25 and 29 kg/m^2 and obesity as a BMI ≥30 kg/m^2. Both conditions are significantly associated with an increased risk of CVD (http://www.escardio.org/guidelines-surveys/esc-guidelines/Pages/cvd-prevention.aspx). This was excluded as a further confounding factor.

Among behavioral factors, CHD subjects were significantly less compliant to OH and oral prevention strategies than control subjects. Participation in an oral health preventive program was characterized by the interval between routine dental visits and professional dental hygiene sessions. These intervals tended to be longer in the CHD group compared with the control group. Furthermore, OH performances seemed to be less consistent and efficacious in the CHD group compared with the control group when analyzing the daily OH frequency and FMPS. A tendency toward poor oral health behaviors by CHD patients has been previously observed (36) in which routine OH and regular dental care were not consistent in CHD patients. A recent survey of 11,869 subjects (37) investigated the association between poor OH and the risk of CVD and among OH, inflammatory markers, and coagulation in a subsample of 4,830 subjects. Poor OH was associated with a higher risk of CVD and increased concentrations of CRP and fibrinogen.

Tooth loss has been implicated as a valid indicator of oral disease history and has been associated with an increased risk of CHD (38–40). An investigation involving 8,000 subjects showed a weak but significant association between missing teeth and CHD, suggesting a common behavioral background of poor self-care and low health prevention awareness (40). Another study showed a higher incidence of CHD among men with fewer than 11 teeth and periodontal disease compared with subjects with an intact dentition (38). It was also suggested that tooth loss may lead to unfavorable changes in diet and a further increase in CHD risk. More recently, data from the Glasgow Alumni Cohort of 12,631 subjects were used to investigate the association between tooth loss and CVD mortality in young adults (ie, 30 years old or younger). Former students with 9 or more missing teeth at baseline had a 35% greater risk of CVD mortality than those with 4 or fewer missing teeth (39). In our study, CHD subjects had over 250% more missing teeth than the control group, showing the tendency to only refer to the dentist in later stages of disease for tooth extraction. The number of missing teeth was statistically significantly associated with CHD in this study.

Chronic periodontitis is associated with atherosclerosis and with an increased prevalence and incidence of CHD independent of the presence of conventional risk factors (8). The National Report from the Italian Society of Periodontology stated that 60% of the population is affected by mild to severe (10%) periodontal disease. In this study, the prevalence of chronic periodontitis was 42.8% in the control group compared with 82.9% in the CHD group, which is dramatically more prevalent than in the general population. Periodontitis was strongly associated with an increased risk of CHD.

AP presents significant similarities with the inflammatory response involved in periodontitis (14). A recent review of epidemiologic studies (41) reported a prevalence of AP ranging between 14% and 70% of all subjects and 0.6% and 8.5% of all teeth, whereas root-filled teeth were evident in 22% to 78% of subjects and 1.3% to 21.5% of all teeth. Furthermore, it was evidenced that AP is approximately 4 times as common in root-filled teeth as in non–root-filled teeth (41). This tendency was confirmed by another study (42) in which the radiographic evidence of root fillings appeared to be the most important risk indicator of AP in the individual. This unfavorable outcome has been associated with poor quality endodontic therapy, which was found in 44%–86% of treated teeth or roots (43–47).

In this study, CHD subjects showed a significantly higher prevalence of AP compared with the control group (85.3% vs 53.5%, respectively) and higher than available epidemiologic data for adults over 18 years of age. Data extrapolated from clinical-based prevalence studies (43, 48–56) suggest a mean number of LEOs per subject ranging between 1 and 1.9. These data were coherent with our findings that healthy controls had a mean LEO of 1.1. However, CHD subjects had a mean LEO of 3.4 per subject, which was significantly different than the control group. As expected, the majority (95%) of LEOs were associated with RCT teeth. A possible, modest association between the incidence of CHD and RCT emerged in previous studies (15, 19) in which RCT was considered a surrogate indicator of pulpal inflammation. However, in this study, no statistically significant differences were found in the number of RCTs between the groups. Subjects with recent RCT (ie, <2 years) were not included in this study because this was assumed to be the minimum time required to assess healing of an endodontic lesion. If lesion healing had still been in progress, the study could have been exposed to the risk of bias. A significant difference was observed between the number of LEOs associated with RCT in the CHD group compared with the control group (85.7% vs 38.5%, respectively). However, because no pretreatment data were available and the evaluation of the overall standards of treatment quality was not the objective of this study, these findings were not correlated with outcomes of therapy.

AP has been associated with an increased risk of CHD. However, the systemic manifestations of periapical inflammatory processes remain controversial. Several studies have shown a significant association between CHD and LEOs through multivariate analysis after adjusting for confounding factors (18, 28, 57). This is contradicted by other studies in which no significant associations were observed (36, 58). In this study, the diagnosis of LEOs alone did not show an evident association with an increased risk of CHD. However, the number of LEOs was strongly associated, and the association was still evident and stable after adjusting for confounding factors such as family history and smoking. The findings show that an increase in the number of LEOs may lead to an increased risk for CHD. This outcome is in concordance with the investigation by Caplan et al (18) and in contrast with Frisk et al (58).

Furthermore, when analyzing the combined prevalence of lesions of periodontal and endodontic origin, 68.2% of subjects in the CHD group were affected by both diseases compared with 21.4% in the control group. Twenty-five percent of subjects in the control group were orally healthy compared with no subjects in the CHD group. This outcome further confirms the poor oral health of CHD subjects compared with control subjects of the same age and area and with a similar socioeconomic profile.

The distribution of the locus for the polymorphism C(−260)T CD14 gene and its susceptibility to ischemic heart disease in different ethnic groups were evaluated by Zhang et al (59). A meta-analysis of 19 studies included a total of 11,813 cases and 6,196 controls. The data were divided into studies from Europe, India, and East Asian countries (China and Japan). The analysis indicated that the prevalence of the genotypes CC, CT, and TT in European populations are 26.8%, 49.6%, and 23.18%, respectively, with a minimum variation in the percentage of these cases compared with controls. This underlines the difficulties in associating the polymorphism C (−260) T CD14 gene with an increased risk of ischemic heart disease in this population.

These studies indicate that the frequency of T at position C(−260)T varies considerably in the control groups of the different ethnic populations studied. Values range from 48.3% in European studies to 56.47% in Indian studies and 51.3% and 47.7% from studies in East Asia reported in English and Chinese, respectively. Furthermore, despite the limitations of this review, a potential association can be observed between the T allele at position C(−260)T CD14 gene and the risk of ischemic heart disease in all populations analyzed. There is greater evidence for such an association in the Asian population.

The aim of this study was to identify a possible association between the CD14 gene promoter polymorphisms at positions −159 and −260 and the different levels of chronic inflammation both in periodontal and endodontic disease. A population of young adults suffering from recent coronary artery disease even in the absence of conventional risk factors was compared with a healthy control group. It is evident that only the proportion of subjects homozygous for the T allele for the polymorphism CD14 C(−260)T is greater than that for the C allele in both the CHD and control groups. No difference was found in the frequency of C or/and T allele in CD14 C(−159)T polymorphism analysis. Although these values were not statistically significant, the frequency of the T allele (calculated as TT + 1/2 CT) for both the analyzed polymorphisms was higher in the CHD and control groups compared with the frequency of allele C. Despite these results, the polymorphism of the CD14 C(−159)T gene and the effect of the T allele of the same polymorphic gene showed a positive association with CHD even after adjusting for the presence of endodontic and periodontal disease. This association was not statistically significant. On the contrary, no associations were found between the studied CD14 polymorphisms, endodontic and periodontal disease, and the risk of CHD for the polymorphism of CD14 C(−260)T.

The stratification required to statistically evaluate the association of polymorphisms with different levels of endodontic and periodontal disease has been unfortunately conditioned by the low sample size. However, the data emerging from this preliminary study are sufficient to stimulate further studies with a larger population. In conclusion, within the limits of this study, middle-aged subjects affected by CHD exhibited a poorer oral health status and a more unfavorable attitude to oral health preventive behaviors than healthy controls with similar demographic and socioeconomic factors. Tooth loss, caries experience, and endodontic and periodontal diseases were significantly associated with an increased risk of CHD. This study shows that a strong association exists between an increased risk for CHD and the number of LEOs. Furthermore, it was possible to identify a typical CHD patient profile as smoker, low compliance to oral preventive strategies, and a higher prevalence of late stage oral diseases. This profile has much to do with lifestyle, suggesting that chronic oral diseases may be considered among unconventional risk factors of CHD.

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The authors deny any conflicts of interest related to this study.

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