Anti-Cardiolipin and Increased Serum Adhesion Molecule Levels in Patients With Aggressive Periodontitis

Harvey A. Schenkein,* Al M. Best,† Carol N. Brooks,* John A. Burmeister,* James A. Arrowood,‡ Michael C. Kontos,‡ and John G. Tew§

Background: We observed that a significant proportion of patients with periodontitis have elevated serum levels of β2-glycoprotein-I–dependent anti-cardiolipin (anti-CL). These prothrombotic autoantibodies, commonly found to be elevated in patients with systemic lupus erythematosus and the antiphospholipid syndrome, are associated with adverse pregnancy outcomes, such as fetal involution, prematurity, and low birth weight, and with cardiovascular sequelae, such as atherosclerosis, stroke, and myocardial infarction. Anti-CL is known to promote vascular inflammation and thrombosis.

Methods: We measured serum levels of markers of vascular inflammation, including soluble intercellular adhesion molecule (sICAM)-1, soluble vascular cell adhesion molecule (sVCAM)-1, and sE-selectin, in 190 subjects with generalized aggressive or chronic periodontitis and in 90 periodontally healthy subjects.

Results: sVCAM-1 and sE-selectin levels were significantly higher in patients with elevated anti-CL (>15 U/ml). This relationship also was observed in the never-smoker subset of subjects, even after correction for demographic and periodontal variables. Within the diagnostic categories, sICAM-1, sVCAM-1, and sE-selectin were significantly higher in generalized aggressive periodontitis patients who had elevated anti-CL compared to those with normal anti-CL. Statistical correction for demographic and periodontal variables indicated that elevated anti-CL remained significantly associated with increased sVCAM-1 and sE-selectin in generalized aggressive periodontitis patients.

Conclusions: Systemic markers of vascular inflammation in patients with aggressive periodontitis are associated with elevated levels of anti-CL. We hypothesize that a subset of periodontitis patients with elevated antiphospholipid antibodies could represent a subgroup at increased risk for obstetrical and cardiovascular sequelae. *J Periodontol 2007;78:459-466.*

KEY WORDS

Anticardiolipin; cell adhesion; inflammation; markers; periodontitis; proteins.

* Clinical Research Center for Periodontal Disease, Virginia Commonwealth University School of Dentistry, Richmond, VA.
† Department of Biostatistics, Virginia Commonwealth University School of Medicine.
‡ Division of Cardiology, Department of Medicine, Virginia Commonwealth University School of Medicine.
§ Department of Microbiology and Immunology, Virginia Commonwealth University School of Medicine.

We determined that a remarkable group of autoantibodies, termed β2-glycoprotein-I (β2GPI)–dependent anti-cardiolipin (anti-CL), are elevated in a subgroup of individuals with generalized periodontitis.[1] Although these antibodies are found in only 1% to 5% of healthy adults,[2] we observed that nearly 20% of periodontitis patients displayed elevated levels of such antibodies. Elevated levels of anti-CL are found frequently in patients with systemic lupus erythematosus (SLE) and in the anti-phospholipid syndrome (APS),[3] with up to 80% of APS patients having elevated immunoglobulin G (IgG) or IgM anti-CL.[4] Notably, these antibodies are associated with clinical sequelae of thrombosis, stroke, myocardial infarction, early atherosclerosis, and adverse pregnancy outcomes, all of which have been suggested to be associated with periodontal infections. It was shown that a variety of microbial pathogens can induce such pathogenic cross-reactive autoantibodies in animals.[5-11]

Studies of patients with SLE and APS indicated that antiphospholipid antibodies are associated with markers of endothelial cell activation and prothrombotic activity.[3,12-17] For example, pathogenic anti-CL in vitro promotes upregulation of endothelial cell intercellular adhesion molecule (ICAM-)1, vascular cell adhesion molecule (VCAM)-1, and E-selectin expression. Furthermore, sera

doi: 10.1902/jop.2007.060305

from SLE patients with elevated levels of anti-CL also contain elevated concentrations of soluble (s) ICAM-1, sVCAM-1, and sE-selectin. It was proposed further that upregulation of E-selectin may mediate some of the prothrombotic pathological effects of anti-CL.[18]

We hypothesized that anti-CL in a subset of periodontitis patients plays an important role in the observed systemic association of periodontitis with stroke, myocardial infarction, and adverse pregnancy outcomes. If this is correct, one would expect that periodontitis patients with elevated anti-CL also would display elevated levels of markers of endothelial cell activation. In this study, we assessed sICAM-1, sVCAM-1, and sE-selectin in periodontitis patients with and without elevated anti-CL and in healthy controls. The results indicated that there are strong associations between elevated anti-CL and markers of systemic vascular inflammation in aggressive periodontitis patients.

MATERIALS AND METHODS

Clinical Methods

This study was approved by the Office of Research Subjects Protection for the Conduct of Human Research of Virginia Commonwealth University. Subjects for this study were recruited through the Virginia Commonwealth University School of Dentistry Clinics between September 1976 and May 2004. The 280 patients selected for this study represented 90 periodontally healthy subjects and 190 periodontitis patients with elevated or normal serum levels of anti-CL. All subjects provided informed consent for the use of their serum samples. All subjects were determined to have no history of diabetes, coronary artery disease, or stroke. Data on smoking history and racial category were determined by self-report; 70 subjects failed to report smoking status. A complete periodontal evaluation, including assessments of probing depth, attachment loss (AL), plaque index,[19] gingival index,[20] bleeding upon probing,[21] and suppuration,[22] was performed on each participant. Measurements were performed at four sites per tooth (mesio-buccal, mid-buccal, disto-buccal, and mid-lingual). At the time of the examination, a blood sample was taken and processed for serum; it was stored at –70°C until utilized.

The subjects were categorized by diagnostic group as follows. Subjects who were periodontally healthy (NP) were those of any age with no evidence of AL or pockets >3 mm, i.e., had no detectable periodontitis. Chronic periodontitis (CP) subjects were >25 years of age with AL ≥2 mm in any extent or severity pattern on more than one tooth, consistent with plaque level and age and no indication of juvenile onset. Generalized aggressive periodontitis (GAgP) subjects were those with a history of disease onset up to age 35 years, with at least eight teeth affected (≥5 mm AL), at least three of which were not first molars and incisors.

Laboratory Methods

All antibodies and antigens were assessed by enzyme-linked immunosorbent assay (ELISA). For determination of IgG and IgM anti-CL, we utilized ELISA kits.‖¶ For each anti-CL assay, an elevated test was defined as >15 units/ml according to the manufacturer. According to the manufacturer, levels of anti-CL in >95% of the normal healthy population are <11.7 U/ml; this result was reproduced in our laboratory for the reported population. Assays for sICAM-1, sVCAM-1, and sE-selectin also used ELISA kits.#**††

Statistical Analyses

For each marker, differences among the groups (periodontal diagnostic categories or elevated versus normal antibody status) were assessed by analysis of variance followed by Tukey's honestly significance difference (HSD) post hoc test. Multivariable analysis of covariance was used to adjust for periodontal diagnostic categories and demographic variables.

RESULTS

Subject Characteristics

Table 1 depicts the clinical and demographic characteristics of the three diagnostic groups. Univariate analyses indicated that significant differences in age, AL, probing depth, and number of teeth were present (P <0.05) and were characteristic of the clinical groups. The distribution of the diagnostic groups by race was different, with white subjects accounting for about half of the periodontitis groups and ~72% of the healthy subject group.

Relationships Between Periodontal Diagnosis and Concentrations of sICAM-1, sVCAM-1, and E-Selectin

Only E-selectin levels were significantly different between the diagnostic groups; sera from the NP subjects contained significantly lower concentrations of sE-selectin compared to CP patients (Table 2). In the subset of patients who were smokers, patients with periodontitis had elevated levels of sVCAM-1 compared to healthy control subjects.

Associations of Elevated Anti-CL With Cell Adhesion Molecules

Anti-CL was associated significantly with sVCAM-1 and sE-selectin levels (Table 3). Subjects with elevated anti-CL had significantly higher serum concentrations

‖ Varelisa Cardiolipin IgG Antibodies, Pharmacia Diagnostics, Kalamazoo, MI.
¶ Varelisa Cardiolipin IgM Antibodies, Pharmacia Diagnostics.
# Human sICAM-1/CD54 Parameter ELISA kit, R&D Systems, Minneapolis, MN.
** Human sVCAM-1/CD106 Quantikine ELISA kit, R&D Systems.
†† Human sE-Selectin/CD62E Parameter ELISA kit, R&D Systems.

Table 1.

Clinical and Demographic Characteristics of the Subject Population

	Chronic Periodontitis (n = 100)	Generalized Aggressive Periodontitis (n = 90)	Healthy (n = 90)	*P* Value
Age (years)*	45.3 ± 9.9	30.1 ± 6.9	35.2 ± 11.0	<0.001
Race	54% white; 46% black	42% white; 58% black	72% white; 28% black	0.0002
Gender (% female)	53	63	55	0.055
AL (mm)*	2.1 ± 1.9	3.1 ± 1.6	0.3 ± 0.3	<0.001
Probing depth (mm)*	2.8 ± 0.8	3.9 ± 1.0	1.9 ± 0.2	<0.0001
Number of teeth*	23.9 ± 6.1	25.7 ± 4.6	27.1 ± 2.3	0.008
% elevated anti-CL	26.0	24.0	15.6	<0.12

* Mean ± SE.

Table 2.

sICAM-1, sVCAM-1, and sE-Selectin Levels in Diagnostic Groups

Diagnostic Group	sICAM-1 ± SE (μg/ml)	sVCAM-1 ± SE (μg/ml)	sE-Selectin ± SE (μg/ml)
All subjects*			
NP	259.4 ± 13.3	659.9 ± 24.6	65.3 ± 3.7
CP	273.5 ± 12.6	682.6 ± 23.3	77.8 ± 3.5†
GAgP	297.7 ± 13.3	661.1 ± 24.6	71.5 ± 3.7
Never-smoking subjects			
NP	256.8 ± 11.2	697.6 ± 26.7	65.3 ± 4.2
CP	233.5 ± 13.7	653.0 ± 32.5	73.3 ± 5.1
GAgP	258.7 ± 25.2	804.4 ± 59.8	88.5 ± 9.4
Currently smoking subjects			
NP	286.7 ± 34.7	560.6 ± 50.6	68.2 ± 8.4
CP	331.8 ± 26.0	724.0 ± 37.8‡	83.3 ± 6.3
GAgP	325.5 ± 32.3	702.0 ± 47.0‡	78.2 ± 7.8

* Includes subjects reported to have been former smokers.
† Significantly greater than NP ($P = 0.05$).
‡ Significantly greater than NP ($P = 0.03$).

of sVCAM-1 and sE-selectin than did subjects with normal levels of anti-CL. Because of literature indicating that smoking influences serum adhesion molecule concentrations and the problems that are inherent in statistically adjusting for the effects of smoking on periodontitis and vascular sequelae, we performed separate analyses on the 122 subjects who reported to never have smoked. No significant associations between periodontal diagnosis and vascular inflammatory marker levels were noted in this group of subjects (Table 2). However, the associations between anti-CL antibody elevation and increased levels of sVCAM-1 and sE-selectin were still noted in the never-smokers, and it remained statistically significant (Table 3). No significant dose relationships between IgG or IgM anti-CL and markers of vascular inflammation were noted. We further observed that associations between anti-CL and serum levels of cell adhesion molecules were not significant if only subjects who currently smoked were considered (Table 3).

Following adjustments for demographic and clinic variables, serum levels of sVCAM-1 and sE-selectin remained associated significantly with anti-CL levels (Table 4). Additionally, sICAM-1 was associated strongly with race; white subjects demonstrated higher adjusted serum levels (290.5 ± 15.5 μg/ml) than black subjects (220.4 ± 18.9 μg/ml; $P = 0.0005$). We also noted that sVCAM-1 was higher in white subjects (813.2 ± 35.8 μg/ml) than in black subjects (601.8 ± 43.6 μg/ml; $P < 0.0001$) and that sE-selectin levels were higher in males (91.7 ± 7.1 μg/ml) than in females (76.2 ± 5.3 μg/ml; $P = 0.02$).

Next, we examined the relationships between anti-CL and cell adhesion molecule levels within the diagnostic groups. As shown in Table 5, there was a strong relationship between elevated anti-CL and higher concentrations of sICAM-1, sVCAM-1, and sE-selectin in the GAgP group. Although only 13 of the 90 subjects with GAgP were never-smokers, and three of those 13 never-smokers had elevated anti-CL, these relationships still held in this small never-smoker subset and were statistically significant (for sVCAM-1, $P = 0.01$; for sE-selectin, $P = 0.02$). The associations of sVCAM-1 and sE-selectin levels with elevated anti-CL were still noted following statistical correction for age, race, gender, probing depth, AL, and number of teeth (Table 6). Further exploration of relationships of anti-CL with cell adhesion molecules in the CP group revealed that there were no significant relationships between any of the reported clinical indices (including mean probing depth, mean AL, mean number

Table 3.

sICAM-1, sVCAM-1, and sE-Selectin Levels in Subjects With Elevated or Normal Anti-CL

Adhesion Molecule	Anti-CL	n	Concentrations (μg/ml)*	*P* Value
All subjects				
sICAM-1	Normal	216	273.1 ± 8.6	0.34
	Elevated	64	289.0 ± 15.8	
sVCAM-1	Normal	216	651.0 ± 15.7	0.02
	Elevated	64	727.0 ± 28.8	
sE-selectin	Normal	216	68.7 ± 2.4	0.007
	Elevated	64	82.2 ± 4.3	
Never-smoking subjects				
sICAM-1	Normal	100	244.9 ± 9.1	0.34
	Elevated	22	265.5 ± 19.4	
sVCAM-1	Normal	100	673.2 ± 21.5	0.03
	Elevated	22	782.6 ± 45.9	
sE-selectin	Normal	100	66.7 ± 3.4	0.006
	Elevated	22	88.7 ± 7.2	
Currently smoking subjects				
sICAM-1	Normal	55	324.2 ± 20.0	0.60
	Elevated	20	303.3 ± 33.8	
sVCAM-1	Normal	55	671.5 ± 30.9	0.77
	Elevated	20	689.0 ± 51.3	
sE-selectin	Normal	55	79.0 ± 5.0	0.70
	Elevated	20	75.3 ± 8.2	

* Mean ± SE.

of teeth) and anti-CL (elevated or normal) or with concentrations of IgG anti-CL or IgM anti-CL.

Finally, we examined associations of serum cell adhesion molecules with individual immunoglobulin isotypes IgG and IgM. For the NP and CP subject groups, no significant associations were found between antibody levels and serum cell adhesion molecule concentrations. However, for the GAgP group, following adjustment for demographic and periodontal variables there were significant associations between IgG anti-CL and sICAM-1 ($P = 0.04$), sVCAM-1 ($P = 0.05$), and sE-selectin ($P = 0.001$). IgM anti-CL was associated significantly with sVCAM-1 ($P = 0.04$).

DISCUSSION

The principal new finding in this study was that aggressive periodontitis patients with elevated anti-CL antibody concentrations were significantly more likely to have higher levels of sICAM-1, sVCAM-1, and sE-selectin. Even following statistical adjustment for demographic and periodontal variables, sVCAM-1 and sE-selectin levels remained associated significantly with anti-CL. Thus, the subset of aggressive periodontitis patients with elevated levels of anti-CL demonstrated an increase in measures of systemic vascular inflammation. Because ~20% of periodontitis patients seem to have elevated serum anti-CL levels,[1] the presence of such antibodies may be a marker for a subset of aggressive periodontitis patients with increased risk for cardiovascular and obstetrical sequelae.

AgP represents a frequently familial subform of periodontitis characterized by early age of onset (often in the late teens and early twenties) and rapidly progressive loss of periodontal attachment.[23] Data from our laboratory indicated that AgP patients demonstrated increased serum concentrations of C-reactive protein,[24] indicating that many of these patients are experiencing a systemic inflammatory response related to their periodontal infection. Although serum cell adhesion molecule concentrations are not elevated in AgP in general (Table 2), a subset of such patients with elevated anti-CL also have elevated levels of sVCAM-1 and sE-selectin (Tables 5 and 6). Although AgP patients have not been studied systematically with respect to systemic sequelae associated with periodontal infection, our data indicated that these patients may be especially susceptible to conditions associated with systemic inflammation.

The observation that a significant proportion of GAgP patients, as well as CP patients, have elevated anti-CL raises the question of the possible source of such antibodies. Anti-CL autoantibodies are found frequently in patients with SLE, and they are characteristic of APS. Recent studies strongly implicated bacterial and viral infections in the etiology of APS because of the induction of cross-reactive anti-CL autoantibodies by microbial pathogens.[5-11] Following production of a series of monoclonal antibodies reactive with β2GPI, Blank et al.[25] identified a hexapeptide (TLRVYK) sequence in β2GPI that is recognized by some anti-β2GPI monoclonal antibodies. A Basic Local Alignment Search Tool (BLAST) database search revealed that several common bacterial and viral pathogens contained peptide sequences with sequence homology to the β2GPI peptide. Furthermore, mice immunized with microbial pathogens, such as *Haemophilus influenzae* or *Neisseria gonorrhea*, that contained homologous sequences related to TLRVYK produced cross-reactive anti-β2GPI. Such antibodies could be isolated by affinity chromatography and induced APS-like symptoms, such as fetal involution, when infused passively into mice. Thus, bacterial infections could lead to the production of pathogenic anti-CL and be responsible for at least a subset of cases of APS. Our observation that patients with periodontitis seemed to have a relatively high prevalence of β2GPI-dependent anti-CL prompted us to search for peptide sequences homologous to TLRVYK in oral

Table 4.

Multiple Regression Analyses of Associations of Study Variables With sICAM-1, sVCAM-1, and E-Selectin Levels in All Never-Smoking Subjects

	sICAM-1		sVCAM-1		sE-Selectin	
Variable	F Ratio	Prob > F	F Ratio	Prob > F	F Ratio	Prob > F
Diagnosis	0.40	0.6669	0.36	0.6956	0.44	0.6414
Age	2.55	0.1130	2.59	0.1101	1.04	0.3083
Race	**12.67**	**0.0005**	**21.72**	**<0.0001**	0.91	0.3408
Gender	0.17	0.6752	1.13	0.2892	**5.50**	**0.0207**
AL	1.55	0.2155	0.77	0.3810	0.39	0.5327
Probing depth	0.36	0.5453	2.46	0.1193	0.07	0.7822
Number of teeth	0.30	0.5828	2.19	0.1408	0.15	0.6960
Anti-CL	2.10	0.1494	**6.97**	**0.0094**	**7.56**	**0.0069**

Prob = probability. **Bold** data highlight statistically significant comparisons.

bacteria. A search revealed that the arg-gingipain (RGP) protease of *Porphyromonas gingivalis* (an important periodontal disease pathogen) may have such sequences (unpublished observation).

The observation that anti-CL is associated with elevated markers of vascular inflammation in aggressive, but not chronic, periodontitis might be explained by the severity and intensity of the exposure of GAgP patients to bacterial antigens and by host response factors that place them at risk for this form of periodontitis. Characteristic of patients with APS is the fact that their anti-CL titers are elevated persistently when tested ≥6 weeks apart. Given the more acute and severe nature of infection in AgP, it might be expected that long-term and persistent systemic exposure to cross-reactive antigens from the periodontal pocket might be more common in aggressive periodontitis than in CP. Furthermore, the underlying genetic factors conferring susceptibility to aggressive periodontitis may relate to the host response to bacterial antigens and have factors in common with susceptibility to APS.

Previous studies demonstrated that smoking influenced serum cell adhesion molecule concentrations and anti-CL levels. The results of studies of serum cell adhesion molecules have been variable. Most reports indicated that there were increased sICAM-1 levels in smokers,[26-28] which decreased with smoking cessation.[26] In contrast, Blann et al.[29] and Mizia-Stec et al.[30] reported no influence of smoking on sICAM-1 levels. Increases in sVCAM-1 levels in smokers have been reported,[29] whereas other investigators demonstrated no effect of smoking on sVCAM-1.[26,30] Lain et al.[28] reported a decrease in sE-selectin levels in smokers, whereas Mizia-Stec et al.[30] reported no change. Fickl et al.[31] reported an increase in anti-CL levels in smokers. The preponderance of data indicated that smoking influenced serum cell adhesion molecule levels and underscores the necessity of demonstrating that concentrations of these inflammatory markers in periodontitis patients are independent of smoking. Our results in a substantial subset of never-smoking subjects demonstrated that associations of anti-CL with these markers was independent of smoking. Furthermore, analyses of these relationships in current smokers revealed that elevated levels of these markers (due to smoking) likely masked the effects of periodontitis on cell adhesion molecule concentrations.

As in prior studies, we were unable to demonstrate in a never-smoking subset of subjects that any of the three markers of vascular inflammation differed between patients with periodontitis and subjects who were periodontally healthy. This result is consistent with that of Glurich et al.,[32] who found no differences in sICAM-1 and sVCAM-1 between periodontal diagnostic groups. Results of multivariate analyses in this patient population further indicated that there are relationships between serum adhesion molecule levels and race and/or gender. The results with respect to racial differences are consistent with those of Miller et al.,[33] who noted that individuals of African origin residing in England had lower levels of sICAM-1, sVCAM-1, and sP-selectin than white subjects.

Antiphospholipids are associated with the elevation of factors associated with dysregulation of endothelial cell function, damage to endothelial cells, and accelerated thrombosis, including sICAM, sVCAM-1,

E-selectin, monocyte chemotactic protein (MCP)-1, matrix metalloproteinase (MMP)-9, tissue plasminogen activator (tPA), sCD40 ligand (sCD154), interleukin-6, and soluble tissue factor (sTF).[13-18,34-40]

Table 5.

sICAM-1, sVCAM-1, and sE-Selectin Levels in Subjects With Elevated or Normal Anti-CL Within Diagnostic Categories

Adhesion Molecule	Anti-CL	n	Concentrations (μg/ml)*	*P* Value
NP subjects				
sICAM-1	Normal	76	259.0 ± 11.4	0.93
	Elevated	14	261.6 ± 26.6	
sVCAM-1	Normal	76	661.1 ± 25.0	0.91
	Elevated	14	654.2 ± 58.3	
sE-selectin	Normal	76	64.4 ± 3.3	0.49
	Elevated	14	70.1 ± 7.6	
CP subjects				
sICAM-1	Normal	74	280.6 ± 16.8	0.41
	Elevated	26	253.1 ± 28.3	
sVCAM-1	Normal	74	676.8 ± 22.5	0.62
	Elevated	26	698.9 ± 38.0	
sE-selectin	Normal	74	77.8 ± 4.6	0.98
	Elevated	26	77.6 ± 7.80	
GAgP subjects				
sICAM-1	Normal	66	280.9 ± 15.4	0.03
	Elevated	24	343.8 ± 25.5	
sVCAM-1	Normal	66	610.6 ± 33.62	0.005
	Elevated	24	800.1 ± 55.7	
sE-selectin	Normal	66	63.3 ± 4.0	0.0002
	Elevated	24	94.2 ± 6.7	

* Mean ± SE.

Furthermore, in vitro examination of the properties of antiphospholipids demonstrated that they induced upregulation of endothelial cell adhesion molecules as well as other mediators of endothelial cell activation. In vitro studies demonstrated that anti-CL can upregulate the expression of sICAM-1, sVCAM-1, sE-selectin, and other inflammatory markers on endothelial cell cultures containing a source of β2GPI.[15,41] The interaction of anti-CL with endothelial monolayers seems to be dependent upon β2GPI, implying an indirect interaction between antibody and endothelial cell surface. Our observation that elevation of anti-CL was associated with increased levels of sVCAM-1, but not sICAM-1, in periodontitis is consistent with the results of Kaplanski et al.[13] for patients with APS. Other studies of APS and SLE demonstrated elevation of sICAM-1 and sVCAM-1.[14,15] Nearly all studies indicated that levels of these cell adhesion molecules rarely are elevated in concert. Similarly, studies of the effects of smoking on serum adhesion molecule levels demonstrated that concentrations of sICAM-1, sVCAM-1, and sE-selectin in serum are regulated independently. Thus, it seems from the present study and many others that the activation of endothelial cells in association with anti-CL or other stimuli does not necessarily result in expression of elevated levels of all classes of cell adhesion molecules in serum. Neither the interactions that lead to increased expression of these molecules nor the factors that regulate their serum concentrations are understood completely.

Our data support the hypothesis that the strong associations between anti-CL and sVCAM-1 and sE-selectin indicate that increases in systemic vascular inflammation in periodontitis patients may occur as a result of an immune response to periodontal disease

Table 6.

Multiple Regression Analyses of Associations of Study Variables With sICAM-1, sVCAM-1, and E-Selectin Levels in Aggressive Periodontitis Patients

	sICAM-1		sVCAM-1		sE-Selectin	
Variable	F Ratio	Prob > F	F Ratio	Prob > F	F Ratio	Prob > F
Age	0.7693	0.3830	0.8780	0.3515	0.0442	0.8339
Race	10.6658	**0.0016**	13.4365	**0.0004**	0.9414	0.3348
Gender	0.1957	0.6594	0.0212	0.8846	2.2841	0.1346
AL	0.1163	0.7339	0.1500	0.6995	0.2287	0.6337
Probing depth	0.3481	0.5568	0.2340	0.6299	0.9434	0.3343
Number of teeth	2.5842	0.1118	0.2941	0.5891	2.4993	0.1177
Anti-CL	3.0716	0.0834	8.9383	**0.0037**	11.9450	**0.0009**

Prob = probability. **Bold** data highlight statistically significant comparisons.

pathogens resulting in the formation of cross-reactive autoantiphospholipid antibodies.

ACKNOWLEDGMENTS

The authors acknowledge the contributions of Kimberly Holloway and Gail Smith, research specialists in the Virginia Commonwealth University Clinical Research Center for Periodontal Disease, for their skillful clinical management of the volunteers who participated in this study. This work was supported by grant DE 13102 from the National Institute of Dental and Craniofacial Research.

REFERENCES

1. Schenkein HA, Berry CR, Burmeister JA, et al. Anticardiolipin antibodies in sera from patients with periodontitis. *J Dent Res* 2003;82:919-922.
2. Petri M. Epidemiology of the antiphospholipid antibody syndrome. *J Autoimmun* 2000;15:145-151.
3. Levine JS, Branch DW, Rauch J. The antiphospholipid syndrome. *N Engl J Med* 2002;346:752-763.
4. Marai I, Gilburd B, Blank M, Shoenfeld Y. Anticardiolipin and anti-beta2-glycoprotein I (beta2GP-I) antibody assays as screening for anti-phospholipid syndrome. *Hum Antibodies* 2003;12:57-62.
5. Sorice M, Pittoni V, Griggi T, et al. Specificity of antiphospholipid antibodies in infectious mononucleosis: A role for anti-cofactor protein antibodies. *Clin Exp Immunol* 2000;120:301-306.
6. Dalekos GN, Zachou K, Liaskos C. The antiphospholipid syndrome and infection. *Curr Rheumatol Rep* 2001;3:277-285.
7. Guglielmone H, Vitozzi S, Elbarcha O, Fernandez E. Cofactor dependence and isotype distribution of anticardiolipin antibodies in viral infections. *Ann Rheum Dis* 2001;60:500-504.
8. Ordi-Ros J, Villarreal J, Monegal F, et al. Anticardiolipin antibodies in patients with chronic hepatitis C virus infection: Characterization in relation to antiphospholipid syndrome. *Clin Diagn Lab Immunol* 2000;7:241-244.
9. Uthman IW, Gharavi AE. Viral infections and antiphospholipid antibodies. *Semin Arthritis Rheum* 2002;31:256-263.
10. Hojnik M, Gilburd B, Ziporen L, et al. Anticardiolipin antibodies in infections are heterogenous in their dependency on beta 2-glycoprotein I: Analysis of anticardiolipin antibodies in leprosy. *Lupus* 1994;3:515-521.
11. Gharavi AE, Pierangeli SS, Harris EN. Origin of antiphospholipid antibodies. *Rheum Dis Clin North Am* 2001;27:551-563.
12. Pierangeli SS, Colden-Stanfield M, Liu X, et al. Antiphospholipid antibodies from antiphospholipid syndrome patients activate endothelial cells in vitro and in vivo. *Circulation* 1999;99:1997-2002.
13. Kaplanski G, Cacoub P, Farnarier C, et al. Increased soluble vascular cell adhesion molecule 1 concentrations in patients with primary or systemic lupus erythematosus-related antiphospholipid syndrome: Correlations with the severity of thrombosis. *Arthritis Rheum* 2000;43:55-64.
14. Pierangeli SS, Espinola RG, Liu X, Harris EN. Thrombogenic effects of antiphospholipid antibodies are mediated by intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and P-selectin. *Circ Res* 2001;88:245-250.
15. Pierangeli SS, Harris EN. Probing antiphospholipid-mediated thrombosis: The interplay between anticardiolipin antibodies and endothelial cells. *Lupus* 2003;12:539-545.
16. Williams FM, Parmar K, Hughes GR, Hunt BJ. Systemic endothelial cell markers in primary antiphospholipid syndrome. *Thromb Haemost* 2000;84:742-746.
17. Ferro D, Pittoni V, Quintarelli C, et al. Coexistence of anti-phospholipid antibodies and endothelial perturbation in systemic lupus erythematosus patients with ongoing prothrombotic state. *Circulation* 1997;95:1425-1432.
18. Espinola RG, Liu X, Colden-Stanfield M, et al. E-Selectin mediates pathogenic effects of antiphospholipid antibodies. *J Thromb Haemost* 2003;1:843-848.
19. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;22:121-135.
20. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1963;21:533-551.
21. Muhlemann HR, Son S. Gingival sulcus bleeding: A leading symptom in initial gingivitis. *Helv Odontol Acta* 1971;15:107-113.
22. Singh S, Cianciola L, Genco RJ. The suppurative index: An indicator of active periodontal disease. *J Dent Res* 1977;56:B200(Abstr. 593).
23. Schenkein HA, Van Dyke TE. Early-onset periodontitis: Systemic aspects of etiology and pathogenesis. *Periodontol 2000* 1994;6:7-25.
24. Salzberg TN, Overstreet BT, Rogers JD, et al. C-reactive protein levels in patients with aggressive periodontitis. *J Periodontol* 2006;77:933-939.
25. Blank M, Krause I, Fridkin M, et al. Bacterial induction of autoantibodies to beta2-glycoprotein-I accounts for the infectious etiology of antiphospholipid syndrome. *J Clin Invest* 2002;109:797-804.
26. Palmer RM, Stapleton JA, Sutherland G, et al. Effect of nicotine replacement and quitting smoking on circulating adhesion molecule profiles (sICAM-1, sCD44v5, sCD44v6). *Eur J Clin Invest* 2002;32:852-857.
27. Keaney JF Jr., Massaro JM, Larson MG, et al. Heritability and correlates of intercellular adhesion molecule-1 in the Framingham Offspring Study. *J Am Coll Cardiol* 2004;44:168-173.
28. Lain KY, Luppi P, McGonigal S, Roberts JM, Deloia JA. Intracellular adhesion molecule concentrations in women who smoke during pregnancy. *Obstet Gynecol* 2006;107:588-594.
29. Blann AD, Seigneur M, Steiner M, Miller JP, McCollum CN. Circulating ICAM-1 and VCAM-1 in peripheral artery disease and hypercholesterolaemia: Relationship to the location of atherosclerotic disease, smoking, and in the prediction of adverse events. *Thromb Haemost* 1998;79:1080-1085.
30. Mizia-Stec K, Zahorska-Markiewicz B, Gasior Z. Cigarette smoking and inflammatory indices in coronary artery disease. *Int J Cardiol* 2004;93:169-174.
31. Fickl H, Van Antwerpen VL, Richards GA, et al. Increased levels of autoantibodies to cardiolipin and oxidised low density lipoprotein are inversely associated

with plasma vitamin C status in cigarette smokers. *Atherosclerosis* 1996;124:75-81.
32. Glurich I, Grossi S, Albini B, et al. Systemic inflammation in cardiovascular and periodontal disease: Comparative study. *Clin Diagn Lab Immunol* 2002;9: 425-432.
33. Miller MA, Sagnella GA, Kerry SM, et al. Ethnic differences in circulating soluble adhesion molecules: The Wandsworth Heart and Stroke Study. *Clin Sci (Lond)* 2003;104:591-598.
34. Cho CS, Cho ML, Chen PP, et al. Antiphospholipid antibodies induce monocyte chemoattractant protein-1 in endothelial cells. *J Immunol* 2002;168:4209-4215.
35. Faber-Elmann A, Sthoeger Z, Tcherniack A, Dayan M, Mozes E. Activity of matrix metalloproteinase-9 is elevated in sera of patients with systemic lupus erythematosus. *Clin Exp Immunol* 2002;127:393-398.
36. Ferro D, Pignatelli P, Loffredo L, et al. Soluble CD154 plasma levels in patients with systemic lupus erythematosus: Modulation by antiphospholipid antibodies. *Arthritis Rheum* 2004;50:1693-1694.
37. George J, Blank M, Levy Y, et al. Differential effects of anti-beta2-glycoprotein I antibodies on endothelial cells and on the manifestations of experimental antiphospholipid syndrome. *Circulation* 1998;97:900-906.
38. Kornberg A, Blank M, Kaufman S, Shoenfeld Y. Induction of tissue factor-like activity in monocytes by anti-cardiolipin antibodies. *J Immunol* 1994;153: 1328-1332.
39. Mo L, Salmon JE. Intercellular adhesion molecule 1 expression is required for antiphospholipid antibody-induced pregnancy loss. *Arthritis Rheum* 2001;44: 1225-1226.
40. Simantov R, Lo SK, Gharavi A, et al. Antiphospholipid antibodies activate vascular endothelial cells. *Lupus* 1996;5:440-441.
41. Lopez-Pedrera C, Buendia P, Barbarroja N, et al. Antiphospholipid-mediated thrombosis: Interplay between anticardiolipin antibodies and vascular cells. *Clin Appl Thromb Hemost* 2006;12:41-45.

Correspondence: Dr. Harvey A. Schenkein, Virginia Commonwealth University School of Dentistry, P.O. Box 980566, Richmond, VA 23298. Fax: 804/828-5787; e-mail: haschenk@vcu.edu.

Accepted for publication September 26, 2006.