

Serum Antibody Levels in Smoker and Non-Smoker Saudi Subjects With Chronic Periodontitis

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Background: Cigarette smoking is a significant risk factor for the initiation and progression of periodontal disease. Studies have shown altered serum and gingival crevicular fluid inflammatory cytokine profiles, immune cell function, and altered proteolytic regulation in smokers. The observations are not consistent, and to date, there is no clear mechanism to explain how smoking may affect periodontal disease. Hence, the present study was undertaken to assess the alterations of serum immunoglobulin levels in smokers with periodontitis and its potential role as a risk indicator of the disease process.

Methods: In this study, 30 patients who smoked and 30 patients who did not smoke with chronic periodontitis and 30 healthy subjects were enrolled. Serum immunoglobulin (Ig) G, IgA, and IgM levels were estimated with immunoturbidimetric assay. The IgG subclass (IgG1, IgG2, IgG3, and IgG4) levels were performed using single radial immunodiffusion assay.

Results: Levels of serum IgG and IgA were significantly lower in smokers compared to non-smokers and healthy controls ($P < 0.001$). Although IgM levels were low in smokers, it was not significant. Of the four subclasses of IgG studied, the IgG2 was found to be significantly lower among smokers with periodontitis.

Conclusions: Current observations indicate that cigarette smoking may be associated with the suppression of B-cell function and immunoglobulin production. The alteration of antibody levels further explains the potential mechanism by which smoking exacerbates periodontal disease. Further studies at the molecular level may highlight the specific mechanism by which tobacco can interact with cells of the immune system and its impact on periodontal disease process. *J Periodontol* 2007;78:1043-1050.

KEY WORDS

Antibodies; immunoglobulin G; immunoglobulins; periodontitis; smoking.

Tobacco smoking has been found to be a major environmental factor associated with generalized forms of severe periodontitis.¹⁻³ The epidemiologic studies⁴⁻⁸ on the relationship between tobacco use and periodontal diseases consistently reported that cigarette smokers are five times more likely to develop severe periodontitis than non-smokers. In a study of young adults with severe periodontal destruction, Mullally et al.⁹ found that subjects with generalized early onset periodontitis smoked more heavily than those with localized forms of this disease. Smoking can affect the pathogenesis of the disease in an individual, change periodontal disease patterns in the population, and affect periodontal therapy outcomes.^{10,11}

Systemic alterations of the cellular and humoral immune responses to periodontal pathogens among smokers have been evaluated, including immunosuppression, exaggerated inflammatory cell responses, impaired neutrophils, and reduced antibody production.^{2,12-14} The involvement of host response to bacteria involved in periodontal disease can be detected by the serum immunoglobulin (Ig) G antibody to particular bacteria and/or their antigens.¹⁵ Immunoglobulin levels of patients with various forms of periodontal diseases have shown that individuals with periodontitis often have elevated total serum IgG. Therefore, it is likely that IgG antibodies play a major protective role in moderating infections and tissue

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destruction caused by periodontal organisms.^{16,17} This elevation in serum immunoglobulins may be caused by the increased antibody production to neutralize the bacterial toxins.^{18,19} The presence or absence of high levels of such antibodies were directly associated with disease severity as indicated by loss of attachment.^{20,21} Kobayashi et al.²² observed significantly higher serum IgG levels among subjects with chronic periodontitis.

Studies^{1,23} assessing the host response to the infection among smokers have shown reduction of many defensive functions. Decreased levels of salivary IgA and serum IgG have been reported among smokers.^{24,25} In particular, serum IgG antibodies to *Prevotella intermedia* and *Fusobacterium nucleatum* have been reported to be reduced in smokers.²⁶ Tangada et al.¹³ examined specific serum antibody concentrations to *Actinobacillus actinomycetemcomitans* in patients with generalized aggressive periodontitis. They observed much lower concentrations of IgG2 antibody in serum from smokers than in non-smokers. Studies^{1,22,27} of IgG subclasses to *Porphyromonas gingivalis* in smokers with chronic periodontitis also showed that IgG2 is depressed. However, serum IgG2 levels in African American subjects are unaffected by smoking, except in generalized aggressive periodontitis.²⁸ In chronic periodontitis, Quinn et al.²⁹ found a reduction in IgG1 and IgG4. Furthermore, Gunsolley et al.³⁰ and Lu et al.³¹ showed that total serum immunoglobulin concentrations are strongly influenced by periodontal disease status and race. An increased concentration of all IgG subclasses were observed in African American compared to white subjects with the same periodontal diagnosis.³² Graswinckel et al.²⁷ found that patients with periodontitis who smoked have lower levels of total IgG and IgG2 than non-smoking patients with periodontitis. This lack of increase in plasma IgG2 levels coincides with increased severity of periodontal destruction in the patients who smoked.

Differences in periodontal disease prevalence, severity, subgingival microflora, and host immune response have been reported for various ethnic/racial groups. Albandar et al.³³ studied the associations of serum concentrations of IgG, IgA, and IgM with aggressive periodontitis and its relation to race. They concluded that race has a significant effect on serum antibody concentrations, irrespective of disease classification, with blacks having significantly higher serum concentrations of IgG1, IgG2, and IgG3 than whites and Hispanics. Serum IgG2 was also significantly elevated in Chinese patients with aggressive periodontitis.³⁴ Craig et al.¹⁶ studied the serum IgG antibody response in three urban minority populations (Asians, African Americans, and Hispanics). The mean serum IgG antibody to *P. gingivalis* was

found to be higher in the African American group, whereas it was lower in the Hispanic group. These results suggest that elevated serum IgG antibody to *P. gingivalis* reflects destructive periodontal disease status and may be considered a risk factor for disease progression in these ethnic/racial populations.

There are inconsistencies and variations in findings reflecting the complex relationship among immunoglobulin subclass, smoking, race/ethnic class, and periodontal disease. The effects of cigarette smoking on total serum IgG, IgA, and IgM classes are controversial, with some reports^{1,27,35} indicating suppression of total antibody titer levels and other studies^{36,37} indicating no effect of smoking on class of total IgG, IgA, and IgM concentrations. These conflicting findings may be related to the differences in the populations studied. The exact mechanism by which tobacco smoking influences the periodontal tissues is still unclear. Therefore, one of the possible biologic explanations for the epidemiologic associations between environmental factors, such as smoking and periodontitis, may be related to modification of antibody production and alteration of circulating immune complexes.^{14,38} Biologic plausible mechanisms for the effects of smoking on periodontium can be described with supportive evidence.

The present study was undertaken to assess the total serum IgG, IgA, and IgM and subclasses of IgG (1, 2, 3, and 4) in smoker and non-smoker Saudi subjects with chronic periodontitis.

MATERIALS AND METHODS

Study Population

A total of 90 systemically healthy, smoker and non-smoker subjects, between 25 and 55 years of age, were enrolled in the study. The subjects included in the test groups were selected from the patients who were referred to the periodontology clinics for diagnosis and treatment of periodontitis from September 2005 to February 2006. The control group was derived from those subjects who attended the restorative dental clinics and from the staff and graduate students of College of Dentistry. Periodontal status of the patients and control subjects was assessed according to the classification of American Academy of Periodontology.³⁹ Smoking status was determined based on daily use.⁴⁰ Approval of the Ethics committee was obtained from the College of Dentistry Research Center (CDRC), King Saud University.

Sixty patients with a periodontal probing depth (PD) ≥ 4 mm and clinical attachment loss (CAL) ≥ 2 mm in $\geq 30\%$ of the teeth were diagnosed as the chronic periodontitis group. Subjects who smoked a minimum of 10 cigarettes/d for ≥ 2 years were included in the smoker periodontitis group (n = 30). The remainder of the patients who never smoked were assigned to

the non-smoker periodontitis group ($n = 30$). Thirty subjects who had clinically healthy gingiva and no CAL (≤ 3 mm periodontal PD) were also included as a healthy group.

An informed consent form was obtained from all subjects enrolled in the study. They were screened clinically, biochemically, and biophysically to exclude subjects with any systemic illness. The following criteria were also used to exclude subjects in the study: 1) age range was <25 or >55 years; 2) subjects had <22 permanent teeth; 3) taking any type of medication chronically or in the last 2 weeks; 4) having any given chronic medical condition, including diabetes and viral, fungal, or bacterial infections; 5) having any type of medical condition in the last 2 weeks, including flu, upper respiratory infection, allergy, skin disorders, sinus problem; 6) receiving any form of physical trauma in the last 2 weeks; 7) having aggressive periodontitis, periodontal abscess, or necrotizing ulcerative gingivitis or periodontitis; 8) received periodontal treatment and/or antibiotic therapy within the preceding 3 months; 9) subjects who had any type of dental work or tooth extraction(s) in the last 2 weeks; 10) having active carious lesions; 11) former smokers; and 12) subjects who refused to sign the consent form.

Clinical Periodontal Examination

An extensive medical history by a written questionnaire and by interview of 20- to 30-minute duration was recorded. For each patient, a set of complete examinations of extraoral and intraoral full-mouth clinical parameters and the individual number of teeth present, excluding the third molars, were documented. One clinical examiner performed all the clinical measurements. Calibration exercises for probing measurements were performed in five patients before the actual study. The intraexaminer agreement was good, with a 0.82 κ value. The following clinical indices, plaque index (PI), bleeding on probing (BOP), periodontal PD, and CAL, were measured at the mesial, distal, buccal, and lingual aspects for each tooth. Smoking history was assessed according to a standardized interview and self-reported questionnaire. The smoking exposure was expressed in terms of consumption (number of cigarettes per day) and duration (years). Former smokers who had quit smoking were excluded.

Venous Blood Samples

Ten milliliters of venous blood sample was collected from each patient by venipuncture in the antecubital fossa without excessive venous stasis. The blood samples were collected using a vacutainer.[†] The samples were allowed to clot, and the serum was separated and centrifuged at 3,000 rpm for 10 minutes. The serum samples were aliquoted and stored in plastic vials at -20°C . Estimation of total serum IgG, IgA,

and IgM levels was performed by immunoturbidimetry. The method is based on assessing the immunoprecipitation reaction by measuring the intensity of transmitted light measured as absorbance with the help of immunoturbidimetric method.^{‡41-44}

Serum levels of IgG subclasses were performed by single radial immunodiffusion (RID). The method involves antigen diffusing radially from a cylindrical well through an agarose gel containing an appropriate mono-specific antibody.^{45,46}

RID Assay

IgG subclass (1, 2, 3, and 4) concentrations were determined with commercially obtained RID kits[§] according to the manufacturer's instructions and using the standards provided with each kit.

Twenty-five microliters of calibrator was mixed with 225 μl 7% bovine serum albumin (BSA) for a 10% dilution. IgG1 and IgG2 test samples were diluted 1/10 (25 μl of each patient sample was mixed with 225 μl 7% BSA). The IgG3 and IgG4 samples did not need dilution. The liquid control serum was diluted for IgG1 and IgG2 samples only in the same way as the test sample. Five microliters of the neat calibrator plus two 5- μl dilutions were used to produce a linear calibration curve. The remaining wells were filled with 5 μl of appropriate test samples and controls. The lid was tightly closed, and the plate was stored flat at room temperature (20°C to 24°C). The plates were incubated for 72 hours. After the required diffusion time, ring diameters were measured to the nearest 0.1 mm, using an RID plate calibrating viewer.^{||} The concentrations of the IgG subclasses were performed and recorded from the plotted calibration curve.

Statistical Analysis

Statistical analysis of data was performed with statistical software.[¶] Means and standard deviations for age, number of teeth, PI, BOP, periodontal PDs, CALs, and serum antibody levels (IgG, IgA, IgM, and IgG subclasses) of the subjects (healthy, non-smoker periodontitis, and smoker periodontitis) were analyzed. Differences among the three study groups for all variables were determined with one-way analysis of variance (ANOVA). When overall ANOVA showed statistical significance, post hoc testing (Tukey-Kramer multiple comparisons test) was performed to explore the differences between any two groups. $P < 0.05$ was considered significant. The student *t* test

[†] Vacutainer, Becton Dickinson, Franklin Lakes, NJ.

[‡] Cobas Integra 800, Roche Diagnostics, F. Hoffmann-La Roche, Basel, Switzerland.

[§] BINDARID Kits, The Binding Site, Birmingham, U.K.

^{||} Transidyne General, Ann Arbor, MI.

[¶] GraphPad InStat, GraphPad Software, San Diego, CA.

was used to analyze the mean differences of periodontal PD and CAL between the two periodontitis groups.

RESULTS

The distribution of subjects according to the age and number of teeth are presented in Table 1. The smoker group had a mean number of 25.80 ± 1.27 teeth present in the mouth at the time of examination. The non-smokers and healthy group had slightly higher numbers of teeth present, with a mean number of 26.30 ± 1.82 teeth and 27.60 ± 0.62 teeth, respectively.

The periodontal PDs and CALs are shown in Table 2. The smoker group had a significantly higher periodontal PD compared to non-smokers ($P < 0.02$). The CAL was significantly higher ($P < 0.03$) among smokers compared to non-smokers.

The mean values of total serum immunoglobulins IgG, IgA, and IgM estimated by the immunoturbidimetric analysis are presented in Table 3. The total serum IgG antibody showed significantly lower levels in the smoker group compared to non-smokers and healthy controls ($P < 0.001$). Smokers had significantly lower IgA antibodies compared to non-smokers and healthy controls ($P < 0.001$). The IgM antibody showed lower levels in smoker periodontitis patients compared to the non-smoker group and healthy controls. Although lower values were observed in the study groups, these were not statistically significant.

Table 1.

Age and Number of Teeth Present Among Smoker Periodontitis, Non-Smoker Periodontitis, and Healthy Groups

Group	Age (years) (mean \pm SD)	N Teeth (mean \pm SD)
Smoker (n = 30)	34.80 ± 8.19	25.80 ± 1.27
Non-smoker (n = 30)	34.57 ± 7.76	26.30 ± 1.82
Healthy (n = 30)	34.53 ± 6.19	27.60 ± 0.62

Table 2.

PD and CAL Between Smoker and Non-Smoker Periodontitis Groups

Group	PD (mm) (mean \pm SD)	CAL (mm) (mean \pm SD)
Smoker (n = 30)	$5.66 \pm 0.41^*$	$3.67 \pm 0.86^\dagger$
Non-smoker (n = 30)	5.43 ± 0.27	3.27 ± 0.59

* Significantly higher than non-smoker group ($P < 0.02$).

† Significantly higher than non-smoker group ($P < 0.03$).

Table 3.

Serum Antibody Levels of IgG, IgA, and IgM Among Smoker Periodontitis, Non-Smoker Periodontitis, and Healthy Groups

Group*	IgG (g/l) (mean \pm SD)	IgA (g/l) (mean \pm SD)	IgM (g/l) (mean \pm SD)
Smoker	$10.66 \pm 1.32^\dagger$	$2.13 \pm 1.00^\dagger$	0.59 ± 0.25
Non-smoker	12.71 ± 1.54	3.11 ± 0.90	0.75 ± 0.38
Healthy	11.36 ± 1.08	2.34 ± 0.70	0.83 ± 0.31

* n = 30 for each group.

† Significantly lower than non-smoker group ($P < 0.001$).

Table 4 shows the mean values of IgG1 to IgG4 for each group studied. The mean of IgG1 serum antibody levels was not significantly different among the three study groups. Serum IgG2 levels were significantly lower in smoker periodontitis patients compared to non-smoker periodontitis patients ($P < 0.001$). In the healthy group, the mean value of IgG2 was 5.08 ± 1.51 g/l. The mean serum level of IgG3 was slightly higher among non-smoker periodontitis patients than smoker periodontitis and healthy groups, whereas the mean differences among the three study groups were not statistically significant. IgG4 was also higher among non-smoker periodontitis patients compared to the smoker periodontitis and healthy groups. Smoker periodontitis patients had lower mean serum levels of IgG4, and differences among the three study groups were not statistically significant.

DISCUSSION

Tobacco smoking seems to be one of the most significant environmental factors in the initiation and progression of destructive periodontal disease.^{4,26,47} Smoking can affect the pathogenesis of periodontal disease and the outcome of periodontal therapy.^{10,48} The exact mechanism by which tobacco exerts its influence on oral health has not been fully understood. Moreover, the temporal sequence of the process is always extremely difficult to explore. However, evidence obtained from cross-sectional risk assessment studies and several longitudinal studies^{49,50} have suggested the causal role of tobacco smoking is the initiation and progression of periodontitis in humans.

There is strong evidence that smoking affects the innate and immune host responses.^{25,51} The effects of cigarette smoking on total serum IgG, IgA, and IgM were reported in several studies.^{1,27,35} It is a fairly consistent finding that smoking decreases serum IgG concentrations.^{14,38} The results of the present study are in agreement with the observations of the previous

Table 4.
Serum Antibody Levels of IgG Subclasses 1, 2, 3, and 4 Among Smoker Periodontitis, Non-Smoker Periodontitis, and Healthy Groups

Group*	IgG1 (g/l) (mean ± SD)	IgG2 (g/l) (mean ± SD)	IgG3 (g/l) (mean ± SD)	IgG4 (g/l) (mean ± SD)
Smoker	7.14 ± 1.64	4.15 ± 1.42 [†]	0.76 ± 0.27	0.34 ± 0.10
Non-smoker	7.23 ± 1.26	6.15 ± 1.06	0.86 ± 0.21	0.37 ± 0.10
Healthy	7.16 ± 1.69	5.08 ± 1.51	0.77 ± 0.25	0.34 ± 0.11

* n = 30 for each group.

[†] Significantly lower than non-smoker group ($P < 0.001$).

researchers.^{1,26-28,52} The serum total IgG was found to be significantly lower among the smokers with periodontitis compared to the non-smokers and the healthy group. One of the mechanisms to explain this finding is that smoking decreases the proliferative capacity of T cells and T cell-dependent antibody responses that affect B-cell function and antibody generation.^{53,54} It is possible that B cells are functionally compromised by the reduced proliferative responses to oral pathogens, resulting in decreased production of serum antibodies.^{52,55} In addition, it has been shown that alveolar macrophages from smokers exhibit reduced expression as antigen-presenting cells.⁵⁶ This may eventually lead to a reduction in the humoral immune response to invading organisms in periodontitis patients.

IgG antibodies have been considered important in preventing periodontal destruction in patients with aggressive and chronic periodontitis.^{28,57} It was observed that serum IgG levels were elevated in individuals with chronic periodontitis.^{22,58,59} The elevated serum immunoglobulin (IgG and IgA) levels in the periodontitis group in the present study are in agreement with the previous studies.^{34,60}

IgG2 is the major immunoglobulin subclass that reacts with bacterial carbohydrates and lipopolysaccharides, and it may serve as a good opsonin. An impairment of IgG2 responses may increase the risk of bacterial disease including periodontal disease.^{28,32,61} Previous studies^{30,62} have shown that smoking modifies the concentration of IgG subclasses. The results of the present study show lower levels of IgG subclasses in smoker periodontitis patients compared to non-smokers. Of the four subclasses studied, IgG2 levels showed significantly lower levels in smokers, which is in agreement with other studies.^{29,30} This could partly be explained by the balance between type 1 and type 2 T-cell response. In smokers with periodontitis, T-cell response is tipping more toward type 2, whereas in non-smokers, a predominantly type 1 T-cell activity

is present. IgG2 production is enhanced by a type 1 T-cell response.⁶³⁻⁶⁵ Graswinckel et al.²⁷ observed that serum IgG2 concentrations are depressed in smokers with chronic periodontitis. The lack of sufficient protective antibodies in smokers (in particular, IgG2 against Gram-negative microorganisms) may be responsible for increased severity of destructive periodontal disease.^{27,66,67}

The current study shows that smoker periodontitis patients have lower levels of total IgA and IgM with a significantly lower concentration of IgA among smokers with periodontitis, which is in agreement with other reports.^{33,35,68,69} IgM antibodies are characteristic of primary immune responses, and many will later switch and lead to production of other antibodies, in particular IgG and IgA, that circulate in the blood and body tissues to provide protection.⁷⁰ Serum IgM levels in the present study did not vary significantly among smokers and non-smokers with periodontitis and healthy subjects, which is in accordance with earlier studies.^{27,33,67}

Because of the relationship between antibodies and periodontal infection, the potential application of antibody titers for periodontal diagnostic purposes has received considerable research attention.^{71,72} Individuals with periodontitis often have elevated antibody titers to periodontal pathogens compared to periodontally healthy controls.^{73,74} The serum IgG and IgG2 antibody may be reflective of the destructive periodontal disease, and its levels can be considered a risk indicator for disease progression.¹⁶ Ethnic/racial differences in host response to bacterial colonization, including serum antibody levels, have also been shown. Studies^{13,29,30} found that, compared to non-Hispanic whites, African Americans had elevated serum antibody to *A. actinomycetemcomitans*. In addition, serum IgG antibody to *P. gingivalis* was found to be higher in African Americans compared to Asians or Hispanic subjects.¹⁶ Taken together, these reports suggest that there are ethnic/racial differences in bacterial colonization and host response, and they are associated with the altered antibody response.

The possible influence of smoking on serum IgG and IgG2 antibody levels noted in the present and previous studies^{1,13,27,29,52} stress the significance of smoking as a potential risk factor for the initiation and progression of periodontitis among smokers. In this study, IgG profiles were reported in a Saudi population and highlight the potential role of smoking on the immune system in reducing the host response to periodontal disease. Further research will elucidate the molecular mechanism by which smoking affects the immune system. Additional controlled, longitudinal studies may expound the significance of serum antibodies as potential markers for periodontal disease.

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REFERENCES

1. Apatzidou DA, Riggio MP, Kinane DF. Impact of smoking on the clinical, microbiological and immunological parameters of adult patients with periodontitis. *J Clin Periodontol* 2005;32:973-983.
2. Martinez-Canut P, Lorca A, Magan R. Smoking and periodontal disease severity. *J Clin Periodontol* 1995;22:743-749.
3. Haber J, Wattles J, Crowley M, Mandell R, Joshipura K, Kent RL. Evidence for cigarette smoking as a major risk factor for periodontitis. *J Periodontol* 1993;64:16-23.
4. Bergstrom J, Preber H. Tobacco use as a risk factor. *J Periodontol* 1994;65(Suppl.):545-550.
5. Haber J, Kent RL. Cigarette smoking in a periodontal practice. *J Periodontol* 1992;63:100-106.
6. Grossi SG, Skrepcinski FB, DeCaro T, Zambon JJ, Cummins D, Genco RJ. Response to periodontal therapy in diabetics and smokers. *J Periodontol* 1996;67(Suppl.):1094-1102.
7. Salvi GE, Lawrence HP, Offenbacher S, Beck JD. Influence of risk factors on the pathogenesis of periodontitis. *Periodontol 2000* 1997;14:173-201.
8. Razali M, Palmer RM, Coward P, Wilson RF. A retrospective study of periodontal disease severity in smokers and non-smokers. *Br Dent J* 2005;198:495-498.
9. Mullally BH, Dace B, Shelburne CE, Wolff LF, Coulter WA. Prevalence of periodontal pathogens in localized and generalized forms of early-onset periodontitis. *J Periodontol Res* 2000;35:232-241.
10. Preshaw PM, Heasman L, Stacey F, Steen N, McCracken GI, Heasman PA. The effect of quitting smoking on chronic periodontitis. *J Clin Periodontol* 2005;32:869-879.
11. Zuabi O, Machtei EE, Ben-Aryeh H, Ardekian L, Peled M, Laufer D. The effect of smoking and periodontal treatment on salivary composition in patients with established periodontitis. *J Periodontol* 1999;70:1240-1246.
12. Tonetti MS. Cigarette smoking and periodontal diseases: Etiology and management of disease. *Ann Periodontol* 1998;3:88-101.
13. Tangada SD, Califano JV, Nakashima K, et al. The effect of smoking on serum IgG2 reactive with *Actinobacillus actinomycetemcomitans* in early-onset periodontitis patients. *J Periodontol* 1997;68:842-850.
14. Palmer RM, Wilson RF, Hasan AS, Scott DA. Mechanisms of action of environmental factors – Tobacco smoking. *J Clin Periodontol* 2005;32(Suppl. 6):180-195.
15. Ebersole JL. Systemic humoral immune responses in periodontal disease. *Crit Rev Oral Biol Med* 1990;1:283-331.
16. Craig RG, Boylan R, Yip J, et al. Serum IgG antibody response to periodontal pathogens in minority populations: Relationship to periodontal disease status and progression. *J Periodontol Res* 2002;37:132-146.
17. Sakai Y, Shimauchi H, Ito HO, Kitamura M, Okada H. *Porphyromonas gingivalis*-specific IgG subclass antibody levels as immunological risk indicators of periodontal bone loss. *J Clin Periodontol* 2001;28:853-859.
18. Lai CH, Listgarten MA, Evian CI, Dougherty P. Serum IgA and IgG antibodies to *Treponema vincentii* and *Treponema denticola* in adult periodontitis, juvenile periodontitis and periodontally healthy subjects. *J Clin Periodontol* 1986;13:752-757.
19. Pazandak DP, Rogers RS 3rd, Reeve CM. T and B lymphocyte distribution in periodontal disease. *J Periodontol* 1978;49:625-630.
20. Ranney RR, Yanni NR, Burmeister JA, Tew JG. Relationship between attachment loss and precipitating serum antibody to *Actinobacillus actinomycetemcomitans* in adolescents and young adults having severe periodontal destruction. *J Periodontol* 1982;53:1-7.
21. Gunsolley JC, Burmeister JA, Tew JG, Best AM, Ranney RR. Relationship of serum antibody to attachment level patterns in young adults with juvenile periodontitis or generalized severe periodontitis. *J Periodontol* 1987;58:314-320.
22. Kobayashi T, Kaneko S, Tahara T, Hayakawa M, Abiko Y, Yoshie H. Antibody responses to *Porphyromonas gingivalis* hemagglutinin A and outer membrane protein in chronic periodontitis. *J Periodontol* 2006;77:364-369.
23. van der Vaart H, Postma DS, Timens W, ten Hacken NH. Acute effects of cigarette smoke on inflammation and oxidative stress: A review. *Thorax* 2004;59:713-721.
24. Bennet KR, Reade PC. Salivary immunoglobulin A levels in normal subjects, tobacco smokers, and patients with minor aphthous ulceration. *Oral Surg Oral Med Oral Pathol* 1982;53:461-465.
25. Barbour SE, Nakashima K, Zhang JB, et al. Tobacco and smoking: Environmental factors that modify the host response (immune system) and have an impact on periodontal health. *Crit Rev Oral Biol Med* 1997;8:437-460.
26. Haber J. Smoking is a major risk factor for periodontitis. *Curr Opin Periodontol* 1994;15:12-18.
27. Graswinckel JE, van der Velden U, van Winkelhoff AJ, Hoek FJ, Loos BG. Plasma antibody levels in periodontitis patients and controls. *J Clin Periodontol* 2004;31:562-568.
28. Quinn SM, Zhang JB, Gunsolley JC, Schenkein JG, Schenkein HA, Tew JG. Influence of smoking and race on immunoglobulin G subclass concentrations in early-onset periodontitis patients. *Infect Immun* 1996;64:2500-2505.
29. Quinn SM, Zhang JB, Gunsolley JC, Schenkein HA, Tew JG. The influence of smoking and race on adult periodontitis and serum IgG2 levels. *J Periodontol* 1998;69:171-177.
30. Gunsolley JC, Pandey JP, Quinn SM, Tew J, Schenkein HA. The effect of race, smoking and immunoglobulin allotypes on IgG subclass concentrations. *J Periodontol Res* 1997;32:381-387.
31. Lu H, Califano JV, Schenkein HA, Tew JG. Immunoglobulin class and subclass distribution of antibodies reactive with the immunodominant antigen of *Actinobacillus actinomycetemcomitans* serotype b. *Infect Immun* 1993;61:2400-2407.
32. Lu H, Wang M, Gunsolley JC, Schenkein HA, Tew JG. Serum immunoglobulin G subclass concentrations in periodontally healthy and diseased individuals. *Infect Immun* 1994;62:1677-1682.
33. Albandar JM, DeNardin AM, Adesanya MR, Winn DM, Diehl SR. Associations of serum concentrations of IgG,

- IgA, IgM and interleukin-1beta with early-onset periodontitis classification and race. *J Clin Periodontol* 2002; 29:421-426.
34. Chung HY, Lu HC, Chen WL, Lu CT, Yang YH, Tsai CC. Immunoglobulin G profiles in different forms of periodontitis. *J Periodontol Res* 2003;38:471-476.
 35. Andersen P, Pedersen OF, Bach B, Bonde GJ. Serum antibodies and immunoglobulins in smokers and non-smokers. *Clin Exp Immunol* 1982;47:467-473.
 36. McSharry C, Banham SW, Boyd G. Effect of cigarette smoking on the antibody response to inhaled antigens and the prevalence of extrinsic allergic alveolitis among pigeon breeders. *Clin Allergy* 1985;15:487-494.
 37. O'Keeffe S, Gzel A, Drury R, Cullina M, Grealley J, Finnegan P. Immunoglobulin G subclasses and spirometry in patients with chronic obstructive pulmonary disease. *Eur Respir J* 1991;4:932-936.
 38. Kinane DF, Peterson M, Stathopoulou PG. Environmental and other modifying factors of the periodontal diseases. *Periodontol 2000* 2006;40:107-119.
 39. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;4:1-6.
 40. Xu L, Loos BG, Craandijk J, Ritsema E, Huffels RA, van der Velden U. Teeth with periodontal bone loss, cigarette smoking and plasma cotinine levels. *J Int Acad Periodontol* 2002;4:39-43.
 41. Becker W, Rapp W, Schwick HG, Storiko K. Methods for the quantitative determination of plasma proteins by immunoprecipitation. *Z Klin Chem Klin Biochem* 1968;6:113-122.
 42. Borque L, Yago M, Mar C, Rodriguez C. Turbidimetry of rheumatoid factor in serum with a centrifugal analyzer. *Clin Chem* 1986;32:124-129.
 43. Blirup-Jensen S. Protein standardization III: Method optimization basic principles for quantitative determination of human serum proteins on automated instruments based on turbidimetry or nephelometry. *Clin Chem Lab Med* 2001;39:1098-1109.
 44. Redondo FL, Bermudez P, Cocco C, et al. Evaluation of Cobas Integra 800 under simulated routine conditions in six laboratories. *Clin Chem Lab Med* 2003; 41:365-381.
 45. Fahey JL, McKelvey EM. Quantitative determination of serum immunoglobulins in antibody-agar plates. *J Immunol* 1965;94:84-90.
 46. Mancini AM, Zampa GA, Vecchi A, Costanzi G. Histoimmunological techniques for detecting anti-insulin antibodies in human sera. *Lancet* 1965;33:1189-1191.
 47. Grossi SG, Zambon JJ, Ho AW, et al. Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *J Periodontol* 1994;65:260-267.
 48. Jansson LE, Hagstrom KE. Relationship between compliance and periodontal treatment outcome in smokers. *J Periodontol* 2002;73:602-607.
 49. Bergstrom J, Eliasson S, Dock J. A 10-year prospective study of tobacco smoking and periodontal health. *J Periodontol* 2000;71:1338-1347.
 50. Bergstrom J, Eliasson S, Preber H. Cigarette smoking and periodontal bone loss. *J Periodontol* 1991;62: 242-246.
 51. Kinane DF, Chestnutt IG. Smoking and periodontal disease. *Crit Rev Oral Biol Med* 2000;11:356-365.
 52. Mooney J, Hodge PJ, Kinane DF. Humoral immune response in early-onset periodontitis: Influence of smoking. *J Periodontol Res* 2001;36:227-232.
 53. Gemmell E, Seymour GJ. Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease. *Periodontol 2000* 2004;35:21-41.
 54. Geng Y, Savage SM, Johnson LJ, Seagrave J, Sopori ML. Effects of nicotine on the immune response. I. Chronic exposure to nicotine impairs antigen receptor-mediated signal transduction in lymphocytes. *Toxicol Appl Pharmacol* 1995;135:268-278.
 55. Savage SM, Donaldson LA, Cherian S, Chilukuri R, White VA, Sopori ML. Effects of cigarette smoke on the immune response. II. Chronic exposure to cigarette smoke inhibits surface immunoglobulin-mediated responses in B cells. *Toxicol Appl Pharmacol* 1991;111:523-529.
 56. Pankow W, Neumann K, Ruschoff J, Schroder R, von Wichert P. Reduction in HLA-DR antigen density on alveolar macrophages of smokers. *Lung* 1991;169: 255-262.
 57. Chen HA, Johnson BD, Sims TJ, et al. Humoral immune responses to *Porphyromonas gingivalis* before and following therapy in rapidly progressive periodontitis patients. *J Periodontol* 1991;62:781-791.
 58. Califano JV, Chou D, Lewis JP, Rogers JD, Best AM, Schenkein HA. Antibody reactive with *Porphyromonas gingivalis* hemagglutinin in chronic and generalized aggressive periodontitis. *J Periodontol Res* 2004;39: 263-268.
 59. Engstrom PE, George M, Larsson P, Lally ET, Taichman NS, Norhagen G. Oral and systemic immunoglobulin G-subclass antibodies to *Actinobacillus actinomycetemcomitans* leukotoxin. *Oral Microbiol Immunol* 1999; 14:104-108.
 60. Booth V, Solakoglu O, Bavisha N, Curtis MA. Serum IgG1 and IgG2 antibody responses to *Porphyromonas gingivalis* in patients with periodontitis. *Oral Microbiol Immunol* 2006;21:93-99.
 61. Whitney C, Ant J, Moncla B, Johnson B, Page RC, Engel D. Serum immunoglobulin G antibody to *Porphyromonas gingivalis* in rapidly progressive periodontitis: Titer, avidity, and subclass distribution. *Infect Immun* 1992; 60:2194-2200.
 62. French M. Serum IgG subclasses in normal adults. *Monogr Allergy* 1986;19:100-107.
 63. Finkelman FD, Katona IM, Mosmann TR, Coffman RL. IFN-gamma regulates the isotypes of Ig secreted during in vivo humoral immune responses. *J Immunol* 1988;140:1022-1027.
 64. Stevens TL, Bossie A, Sanders VM, et al. Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature* 1988;334:255-258.
 65. Finkelman FD, Goroff DK, Fultz M, Morris SC, Holmes JM, Mond JJ. Polyclonal activation of the murine immune system by an antibody to IgD. X. Evidence that the precursors of IgG1-secreting cells are newly generated membrane IgD+B cells rather than the B cells that are initially activated by anti-IgD antibody. *J Immunol* 1990;145:3562-3569.
 66. Kinane DF, Mooney J, MacFarlane TW, McDonald M. Local and systemic antibody response to putative periodontopathogens in patients with chronic periodontitis: Correlation with clinical indices. *Oral Microbiol Immunol* 1993;8:65-68.
 67. Albandar JM, DeNardin AM, Adesanya MR, Diehl SR, Winn DM. Associations between serum antibody levels to periodontal pathogens and early-onset periodontitis. *J Periodontol* 2001;72:1463-1469.
 68. Myint MM, Steinsvoll S, Odden K, Dobloug J, Schenck K. Salivary IgA responses to bacteria in dental plaque

- as related to periodontal and HIV infection status. *Eur J Oral Sci* 1997;105:562-570.
69. Anil S, Remani P, Vijayakumar T, Hari S. Cell-mediated and humoral immune response in diabetic patients with periodontitis. *Oral Surg Oral Med Oral Pathol* 1990;70:44-48.
70. Staines NA, Brostoff J, James K. *Introducing Immunology*, 2nd ed. London: Mosby; 1993:50-54.
71. Pietrzak ER, Polak B, Walsh LJ, Savage NW, Seymour GJ. Characterization of serum antibodies to *Porphyromonas gingivalis* in individuals with and without periodontitis. *Oral Microbiol Immunol* 1998;13:65-72.
72. Pussinen PJ, Vilkkuna-Rautiainen T, Alftan G, Mattila K, Asikainen S. Multiserotype enzyme-linked immunosorbent assay as a diagnostic aid for periodontitis in large-scale studies. *J Clin Microbiol* 2002;40:512-518.
73. Kinane DF, Lappin DF, Koulouri O, Buckley A. Humoral immune responses in periodontal disease may have mucosal and systemic immune features. *Clin Exp Immunol* 1999;115:534-541.
74. Ebersole JL, Taubman MA, Smith DJ, Frey DE, Haffajee AD, Socransky SS. Human serum antibody responses to oral microorganisms. IV. Correlation with homologous infection. *Oral Microbiol Immunol* 1987;2:53-59.

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