Hyperhomocysteinemia and the Development of Chronic Kidney Disease in a General Population: The Hisayama Study

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• ***Background:*** **Hyperhomocysteinemia has been linked with various atherosclerotic diseases, but has not been evaluated sufficiently as a risk factor for the development of chronic kidney disease (CKD) in the general population.** ***Methods:*** **To clarify this issue, we followed up 1,477 community-dwelling individuals without CKD, aged 40 years or older, for 5 years and examined the effects of baseline serum total homocysteine (tHcy) levels on the development of CKD.** ***Results:*** **During follow-up, 88 subjects experienced CKD. Baseline tHcy levels were greater in men than women (1.35 versus 1.04 mg/L [10.0 versus 7.7 μmol/L]; $P < 0.01$). Age-adjusted 5-year incidences were 2.2% in the low tertile, 5.4% in the middle tertile, and 8.6% in the high tertile of tHcy levels for men and 3.3%, 6.0%, and 6.9% for women, respectively. The difference between the low and high tertiles was statistically significant for both sexes ($P < 0.05$). In multivariate analysis, these relationships remained substantially unchanged, even after adjustment for other confounding factors, such as systolic blood pressure, antihypertensive medication, hemoglobin A_{1c} level, total cholesterol level, high-density lipoprotein cholesterol level, habitual smoker status, regular alcohol intake, proteinuria, and baseline kidney function (odds ratio [OR] in the high tertile of tHcy levels, 2.09; 95% confidence interval [CI], 0.66 to 6.61 for men; OR, 2.86; 95% CI, 1.10 to 7.43 for women). Furthermore, baseline tHcy level showed a significantly inverse association with rate of change in kidney function during the 5 years after being adjusted for confounding factors, including baseline kidney function.** ***Conclusion:*** **Our findings suggest that elevated serum tHcy levels are a significant risk factor for the development of CKD in the general population.** ***Am J Kidney Dis*** **44:437-445.**

INDEX WORDS: Homocysteine; chronic kidney disease (CKD); glomerular filtration rate (GFR); prospective study; general population.

CHRONIC KIDNEY DISEASE (CKD) is a worldwide public health problem; there is an increasing incidence and prevalence of renal failure, with poor outcomes and high cost.[1] Thus, treatment of CKD at earlier stages is of significance in preventing the progression toward renal failure.[2-4]

Identification of factors responsible for the progression toward renal failure is an ongoing area of interest. Several previous studies reported that age, blood pressure, diabetes, proteinuria, such dyslipidemia as apolipoprotein B or high-density lipoprotein (HDL) cholesterol level abnormalities, and smoking were associated with the subsequent decline in glomerular filtration rate (GFR).[5-9] However, regardless of the treatment and prevention of these factors, patients with renal failure are increasing in number, suggesting that other factors also must be evaluated.

Homocysteine is a sulfur-containing amino acid formed during metabolism of the essential amino acid methionine.[10] In 1969, elevated plasma total homocysteine (tHcy) levels were first linked with vascular disease by McCully.[11] Recent studies have confirmed that elevated tHcy levels are associated with atherosclerotic disease in coronary, cerebral, and peripheral blood vessels in the general population,[12-23] similar to the tendency observed in patients with renal failure.[24,25] Thus, it is reasonable to hypothesize that hyperhomocysteinemia may lead to intrarenal arteriosclerotic lesions and decline in GFR. However, previous prospective studies examining subjects without diabetes with reduced GFR could not find a significant relationship between hyperhomocysteinemia and decline in renal function.[26,27] Furthermore, this issue has not yet been examined in any prospective cohort studies of subjects with normal renal function.

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Received January 8, 2004; accepted in revised form May 20, 2004.

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0272-6386/04/4403-0006$30.00/0
doi:10.1053/j.ajkd.2004.05.024

To clarify whether hyperhomocysteinemia has a significant role in deterioration in renal function, we conducted a prospective cohort study in a Japanese community and investigated the relationship between moderate hyperhomocysteinemia and the development of CKD in subjects with normal renal function.

METHODS

Study Population

The Hisayama study, an epidemiological study of cerebrovascular and cardiovascular diseases, was established in 1961 in Hisayama Town, a suburban community adjacent to Fukuoka City, a metropolitan area of Kyushu Island in southern Japan. The population of the town is approximately 7,500, and full community surveys of the residents have been repeated since 1961.[28]

In 1988, a screening survey for the present study was performed in Hisayama Town. A detailed description of this survey was published previously.[29] Briefly, 2,736 Hisayama residents aged 40 years or older (80.7% of the total population of this age group) consented to participate in the examination and underwent a comprehensive assessment, including estimation of GFR. After excluding 1 subject for whom no blood sample was obtained, 110 subjects with frozen blood samples inadequate for measuring serum tHcy levels, and 324 subjects with moderate or severe CKD (GFR $<$ 60 mL/min/1.73 m^2), the remaining 2,301 individuals were enrolled in this study. Of those, 1,477 subjects (596 men, 881 women) who participated in the health examination in 1993 were finally determined to be the cohort for the present study (reparticipation rate, 64.1%).

Risk Factors

At baseline examination, a self-administered questionnaire concerning current antihypertensive treatment, habitual smoker status, and regular alcohol intake was completed in advance by each participant and checked by trained interviewers at the screening. These variables were classified as being either habitually used or not. Blood pressure was measured 3 times after subjects had rested for at least 5 minutes by using a standard mercury sphygmomanometer with subjects in a sitting position. The mean of the 3 measurements was used for the analysis. Body height and weight were measured in light clothing without shoes, and body mass index was calculated as weight in kilograms divided by height in meters squared. Study physicians performed physical examinations on all participants and rechecked their medical histories.

Blood samples were collected from an antecubital vein after an overnight fast for determination of serum creatinine, urea nitrogen, albumin, lipid, and plasma glucose levels. These specimens were assayed within 24 hours. Part of the serum was stored at -20°C until measurement of tHcy. Fresh-voided urine samples were collected at the examination, and proteinuria is defined as 1^+ or more by using a reagent strip. Serum creatinine concentration was measured by Jaffé's method, and plasma fasting glucose concentration was measured by means of the glucose oxidase method. Hemoglobin A_{1c} level was measured by means of high-performance liquid chromatography. Total cholesterol and HDL cholesterol levels were determined enzymatically. Frozen serum samples were thawed and assayed for serum tHcy using the high-performance liquid chromatography method in 2002.

Definition of CKD and GFR Slope

GFR was estimated using the simplified prediction equation derived from the Modification of Diet in Renal Disease (MDRD) study[30] and derived using the following equation:

$$\text{GFR (mL/min/1.73 m}^2) = 170 \times (\text{serum creatinine [mg/dL]})^{-0.999} \times (\text{age [years]})^{-0.176} \times (\text{serum urea nitrogen [mg/dL]})^{-0.170} \times (\text{serum albumin [g/dL]})^{+0.318} \times (0.762 \text{ if female})$$

GFR less than 60 mL/min/1.73 m^2 ($<$1.00 mL/s/1.73 m^2) is defined as CKD according to the National Kidney Foundation–Kidney Disease Outcomes Quality Initiative guidelines.[31]

The rate of change in GFR was calculated as GFR slope by using the following equation:

$$\text{GFR slope (mL/min/1.73 m}^2\text{/y)} = (\text{GFR in 1993 [mL/min/1.73m}^2] - \text{GFR in 1988 [mL/min/1.73 m}^2])/5$$

Statistical Analysis

The SAS computer package (SAS Institute, Cary, NC)[32] was used to perform all statistical analyses. To analyze serum tHcy levels as categorical variables, they were divided into tertiles according to sex. The median value of tHcy levels in each tertile was used as the categorical value for each level. Relationships between tHcy levels and relevant factors or GFR slope were tested by means of linear regression or logistic regression analysis, as appropriate. In these analyses, serum tHcy and creatinine levels were transformed into logarithms to improve the skewed distribution. Age-adjusted cumulative incidences of CKD were calculated by means of the direct method using the World Health Organization standard population in 1998 and compared by means of Mantel-Haenszel chi-square test using 10-year age groupings. Age- and multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) also were calculated by means of logistic regression analysis. P less than 0.05 is considered statistically significant in all analyses.

RESULTS

Baseline characteristics of subjects according to sex are listed in Table 1. Mean age was 58 years for men and 57 years for women. Mean GFR, serum urea nitrogen level, and creatinine level and frequency of proteinuria were greater

Table 1. Clinical Characteristics of Study Population According to Sex in 1988

Variables	Men (n = 596)	Women (n = 881)
Age (y)	58 ± 10	57 ± 10
GFR (mL/min/1.73 m^2)	78.7 ± 11.0	73.0 ± 8.7
Serum urea nitrogen (mg/dL)	16 ± 4	15 ± 3
Creatinine (mg/dL)	1.1 ± 0.1	0.9 ± 0.1
Proteinuria (%)	7.7	3.2
Albumin (g/dL)	4.3 ± 0.2	4.3 ± 0.2
Systolic blood pressure (mm Hg)	134 ± 20	130 ± 20
Diastolic blood pressure (mm Hg)	80 ± 11	76 ± 11
Antihypertensive medication (%)	12.8	11.5
Regular alcohol intake (%)	60.9	8.7
Habitual smokers (%)	46.5	5.1
Fasting blood glucose (mg/dL)	106 ± 23	102 ± 21
Hemoglobin A_{1c} (%)	5.6 ± 0.7	5.5 ± 0.7
Total cholesterol (mg/dL)	199 ± 40	214 ± 40
HDL cholesterol (mg/dL)	49 ± 11	52 ± 11
Body mass index (kg/m^2)	22.9 ± 2.8	23.1 ± 3.2
tHcy (μmol/L)	10.0 ± 3.6	7.7 ± 2.2

NOTE. Values expressed as mean ± SD or frequencies. To convert GFR in mL/min to mL/s, multiply by 0.01667; serum urea nitrogen in mg/dL to mmol/L, multiply by 0.357; serum creatinine in mg/dL to μmol/L, multiply by 88.4; albumin in g/dL to g/L, multiply by 10; glucose in mg/dL to mmol/L, multiply by 0.05551; total or HDL cholesterol in mg/dL to mmol/L, multiply by 0.02586; tHcy in μmol/L to mg/L, divide by 7.397.

in men than women, whereas mean values for albumin were not different between sexes. Mean systolic and diastolic blood pressures and frequency of antihypertensive medication use also were greater in men. Regular alcohol intake and habitual smoker status were much more frequent in men than women. Male subjects had greater mean fasting plasma glucose and hemoglobin A_{1c} levels. Mean values for total cholesterol were greater in women, whereas mean HDL cholesterol level and body mass index were similar for both sexes. Men had greater serum tHcy levels than women.

Mean values or frequencies of potentially relevant factors are listed by tertiles of serum tHcy levels for men in Table 2. Mean age was not significantly different among serum tHcy levels. Mean values for GFR significantly decreased with increasing tHcy levels, but opposite effects were observed for mean creatinine and albumin levels and frequency of proteinuria. Mean serum urea nitrogen levels did not change with changing tHcy levels. Mean systolic blood pressure showed a tendency to increase with increasing serum tHcy levels, but mean diastolic blood pressure and frequency of antihypertensive medication did not. The frequency of regular alcohol intake significantly decreased with increasing tHcy levels. No dose-response relationships were observed between tHcy levels and frequency of habitual smoker status or mean values for fasting blood glucose, hemoglobin A_{1c}, total cholesterol, and HDL cholesterol, whereas mean body mass index significantly increased with increasing tHcy levels.

Women showed the same tendencies as men for all except 6 relevant factors: mean age and frequency of antihypertensive medication significantly increased with elevating tHcy levels, whereas the opposite effect was observed for mean HDL cholesterol level. Frequency of proteinuria, alcohol intake, and mean body mass index did not change with changing tHcy levels (Table 3).

During the 5-year follow-up, 88 subjects (39 men, 49 women) experienced CKD. Age-adjusted 5-year cumulative incidence rates of CKD according to tertiles of serum tHcy levels are shown according to sex in Fig 1. The incidence was 2.2% in the low tertile, 5.4% in the middle tertile, and 8.6% in the high tertile of tHcy levels for men and 3.3%, 6.0%, and 6.9% for women, respectively. The difference between low and high tertiles was statistically significant for both sexes ($P < 0.05$). However, mean values for GFR at the end of follow-up in subjects who developed CKD were not significantly different among tHcy tertiles in each sex (mean GFR, 55 ± 4 [SD] mL/min/1.73 mm^2 [0.92 ± 0.06 mL/s/1.73 mm^2] in the low tertile, 55 ± 5 mL/min/1.73 mm^2 [0.91 ± 0.09 mL/s/1.73 mm^2] in the middle tertile, and 53 ± 11 mL/min/1.73 mm^2 [0.88 ± 0.18 mL/s/1.73 mm^2] in the high tertile of tHcy levels for men; 56 ± 4 mL/min/1.73 mm^2 [0.94 ± 0.07 mL/s/1.73 mm^2], 55 ± 5 mL/min/1.73 mm^2 [0.91 ± 0.09 mL/s/1.73 mm^2], and 57 ± 2 mL/min/1.73 mm^2 [0.95 ± 0.04 mL/s/1.73 mm^2] for women, respectively).

As shown in model 1 in Table 4, age-adjusted logistic analysis showed that risk for CKD increased with increasing tHcy levels in both men (middle tertile: OR, 2.27; 95% CI, 0.78 to 6.63; high tertile: OR, 3.68; 95% CI, 1.32 to 10.23) and women (middle tertile: OR, 2.34; 95% CI,

Table 2. Mean Values or Frequencies of Potential Risk Factors and Laboratory Variables According to Tertiles of tHcy Levels for 596 Men in 1988

Variables	Tertiles of tHcy Levels (μmol/L)			
	≤8.3 (n = 187)	8.4-10.5 (n = 210)	≥10.6 (n = 199)	*P* for Trend
Age (y)	57 ± 10	59 ± 10	59 ± 11	NS
GFR (mL/min/1.73 m^2)	82 ± 11	78 ± 11	76 ± 10	<0.01
Serum urea nitrogen (mg/dL)	15 ± 4	16 ± 4	16 ± 3	NS
Creatinine (mg/dL)	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	<0.01
Proteinuria (%)	4.8	6.7	11.6	<0.05
Albumin (g/dL)	4.2 ± 0.3	4.3 ± 0.2	4.3 ± 0.2	<0.05
Systolic blood pressure (mm Hg)	131 ± 19	134 ± 17	135 ± 23	<0.05
Diastolic blood pressure (mm Hg)	79 ± 11	81 ± 11	80 ± 12	NS
Antihypertensive medication (%)	9.1	13.8	15.1	NS
Regular alcohol intake (%)	69.0	58.1	56.3	<0.05
Habitual smokers (%)	54.0	41.0	45.2	NS
Fasting blood glucose (mg/dL)	106 ± 24	107 ± 24	105 ± 19	NS
Hemoglobin A_{1c} (%)	5.7 ± 0.7	5.6 ± 0.7	5.6 ± 0.7	NS
Total cholesterol (mg/dL)	196 ± 37	204 ± 39	195 ± 42	NS
HDL cholesterol (mg/dL)	49 ± 11	50 ± 11	47 ± 11	NS
Body mass index (kg/m^2)	22.5 ± 2.8	23.1 ± 2.8	23.2 ± 2.9	<0.01

NOTE. Values expressed as mean ± SD or frequencies. To convert GFR in mL/min/1.73 m^2 to mL/s/1.73 m^2, multiply by 0.01667; serum urea nitrogen in mg/dL to mmol/L, multiply by 0.357; serum creatinine in mg/dL to μmol/L, multiply by 88.4; albumin in g/dL to g/L, multiply by 10; fasting blood glucose in mg/dL to mmol/L, multiply by 0.05551; total or HDL cholesterol in mg/dL to mmol/L, multiply by 0.02586; tHcy in μmol/L to mg/L, divide by 7.397.

Abbreviation: NS, not significant.

0.91 to 6.05; high tertile: OR, 2.93; 95% CI, 1.16 to 7.39). These relationships remained substantially unchanged, even after adjustment for other confounding factors, such as systolic blood pressure, antihypertensive medication, hemoglobin A_{1c} level, total cholesterol level, HDL cholesterol level, serum albumin level, regular alcohol intake, habitual smoker status, and proteinuria (model 2). ORs in the high tertile were 3.42 (95% CI, 1.15 to 10.20) for men and 3.20 (95% CI, 1.25 to 8.22) for women. When baseline inverse serum creatinine was added to the independent variables used in model 2, risk for CKD in the high tertile of tHcy levels tended to increase for men, but not significantly, probably because of overadjustment (model 3). These relationships remained unchanged after adjusting for GFR calculated using the MDRD formula or for creatinine clearance calculated using the Cockcroft-Gault formula, rather than inverse serum creatinine.

Finally, we performed a slope analysis in which the association between continuous values of tHcy and GFR slope was examined by using a multiple regression model after adjusting for age, systolic blood pressure, antihypertensive medication, hemoglobin A_{1c} level, total cholesterol level, HDL cholesterol level, serum albumin level, habitual smoker status, regular alcohol intake, proteinuria, and inverse serum creatinine. This analysis showed a significantly negative association between tHcy levels and rates of change in GFR for both sexes (for GFR slope by an increment of 1 log of tHcy concentration: β, -0.63; F value, 5.91; $P < 0.05$ for men; β, -0.73; F value, 8.18; $P < 0.01$ for women).

DISCUSSION

To our knowledge, this is the first population-based cohort study showing that serum tHcy levels are positively associated with the development of CKD. This association remained unchanged, even after adjustments were made for other confounding factors, such as age, sex, systolic blood pressure, antihypertensive medication, hemoglobin A_{1c} level, total cholesterol level, HDL cholesterol level, regular alcohol intake, habitual smoker status, proteinuria, and baseline renal function, suggesting that moderately el-

Table 3. Mean Values or Frequencies of Potential Risk Factors and Laboratory Variables According to Tertiles of tHcy Levels for 881 Women in 1988

Variables	Tertiles of tHcy Levels (μmol/L) ≤6.6 (n = 276)	6.7-8.2 (n = 311)	≥8.3 (n = 294)	*P* for Trend
Age (y)	55 ± 10	57 ± 10	60 ± 11	<0.01
GFR (mL/min/1.73 m^2)	75 ± 9	73 ± 8	72 ± 9	<0.01
Serum urea nitrogen (mg/dL)	14 ± 3	14 ± 3	15 ± 3	NS
Creatinine (mg/dL)	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	<0.01
Proteinuria (%)	3.6	3.2	2.7	NS
Albumin (g/dL)	4.2 ± 0.2	4.2 ± 0.2	4.3 ± 0.2	<0.05
Systolic blood pressure (mm Hg)	127 ± 20	129 ± 19	134 ± 20	<0.01
Diastolic blood pressure (mm Hg)	75 ± 12	76 ± 10	76 ± 10	NS
Antihypertensive medication (%)	8.3	11.3	14.6	<0.05
Regular alcohol intake (%)	9.8	8.7	7.8	NS
Habitual smokers (%)	5.4	4.2	5.8	NS
Fasting blood glucose (mg/dL)	102 ± 25	101 ± 21	101 ± 17	NS
Hemoglobin A_{1c} (%)	5.5 ± 0.9	5.5 ± 0.6	5.5 ± 0.6	NS
Total cholesterol (mg/dL)	212 ± 40	215 ± 40	217 ± 40	NS
HDL cholesterol (mg/dL)	53 ± 12	51 ± 11	51 ± 11	<0.05
Body mass index (kg/m^2)	23.0 ± 3.2	22.9 ± 3.1	23.3 ± 3.2	NS

NOTE. Values expressed as mean ± SD or frequencies. To convert GFR in mL/min/1.73 m^2 to mL/s/1.73 m^2, multiply by 0.01667; serum urea nitrogen in mg/dL to mmol/L, multiply by 0.357; serum creatinine in mg/dL to μmol/L, multiply by 88.4; albumin in g/dL to g/L, multiply by 10; fasting blood glucose in mg/dL to mmol/L, multiply by 0.05551; total or HDL-cholesterol in mg/dL to mmol/L, multiply by 0.02586; tHcy in μmol/L to mg/L, divide by 7.397.

Abbreviation: NS, not significant.

evated serum tHcy levels are an independent risk factor for CKD in the general population.

Previously, 2 prospective studies investigated the relationships between hyperhomocysteinemia and progression of kidney disease. Samuelsson et al[26] did not find a significant relationship between tHcy level and GFR decline in a follow-up study of 83 patients without diabetes with reduced GFR. Similar findings were observed in another follow-up study of 804 patients without diabetes with moderate or severe decline in GFR.[27] These inconsistent results may be caused by such method limitations as inadequate statistical power because of the small number of subjects and short follow-up periods (within 3.5 years) in these previous studies. Another possible reason for the discrepancy between the previous studies and ours is that effects of hyperhomocysteinemia may differ between subjects with normal renal function and those with reduced renal function; presumably relevant factors, such as blood pressure and proteinuria, affect the progression of kidney disease more strongly than tHcy level in subjects with a moderate or severe decline in GFR.

Several mechanisms by which tHcy might cause vascular damage have been suggested. Elevated tHcy levels promote the proliferation of vascular smooth muscle cells by stimulation of the mitogen-activated protein kinase signal transduction pathway and DNA synthesis.[10] They also impair endothelial vasodilatation by inhibiting the generation of endothelial mediators, including nitric oxide, and they promote adhesion between neutrophil and endothelial cells.[10] tHcy generates superoxide radicals, which inhibit the endothelial-dependent relaxation of vessels.[33] Furthermore, tHcy oxidizes low-density lipoprotein and thus may promote the cellular uptake of modified low-density lipoprotein.[34] Through these mechanisms, elevated tHcy levels might cause intrarenal arteriosclerosis or arteriolar hyalinosis, resulting in a chronic reduction in renal perfusion pressure.[10,35] Chronic hypoperfusion leads to focal or global glomerulosclerosis, tubular atrophy, and interstitial fibrosis.[35] In our previous autopsy-based population survey, intrarenal arteriosclerosis, arteriolar hyalinosis, and glomerulosclerosis were associated closely with reduced GFR.[36] Thus, it is conceivable that el-

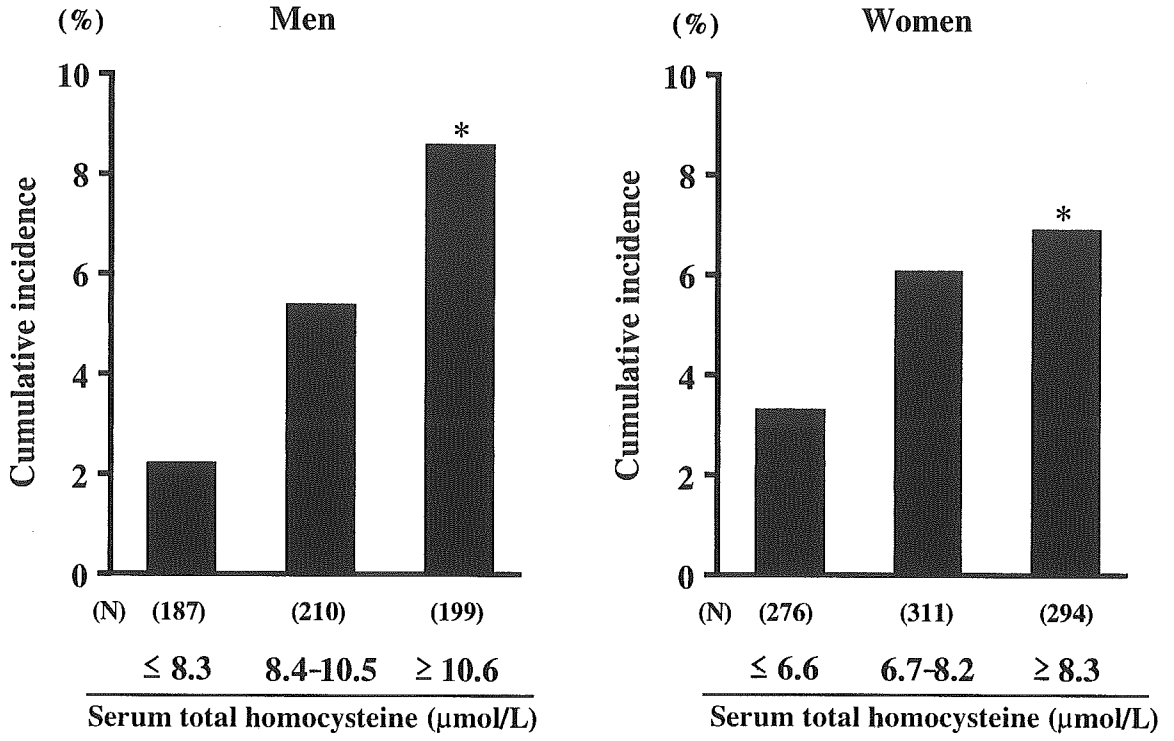

Fig 1. Age-adjusted 5-year cumulative incidence rates of CKD according to tertiles of tHcy levels by sex. *$P <$ 0.05 versus low tertile. To convert tHcy in μmol/L to mg/L, divide by 7.397.

evated tHcy levels induce the progression of intrarenal arteriosclerotic vascular lesions, resulting in the development of CKD.

Previous prospective and cross-sectional studies have shown clear associations between tHcy level and the development of coronary heart disease and stroke,[12-23] as well as the anatomic extent of systemic atherosclerosis.[37,38] Furthermore, in recent cross-sectional and prospective studies of general populations, elevated tHcy

Table 4. Multivariate-Adjusted Analysis for the Development of CKD According to Tertiles of tHcy Levels by Sex During a 5-Year Follow-Up

	Tertiles of tHcy Levels (μmol/L)			
	≤8.3 (n = 187)	8.4-10.5 (n = 210)	≥10.6 (n = 199)	*P* for Trend
Men				
Model 1	1.00	2.27 (0.78-6.63)	3.68 (1.32-10.23)*	<0.01
Model 2	1.00	2.28 (0.74-7.02)	3.42 (1.15-10.20)*	<0.05
Model 3	1.00	1.68 (0.52-5.42)	2.09 (0.66-6.61)	0.23
	≤6.6 (n = 276)	6.7-8.2 (n = 311)	≥8.3 (n = 294)	
Women				
Model 1	1.00	2.34 (0.91-6.05)	2.93 (1.16-7.39)*	<0.05
Model 2	1.00	2.52 (0.96-6.61)	3.20 (1.25-8.22)*	<0.05
Model 3	1.00	2.37 (0.90-6.25)	2.86 (1.10-7.43)*	<0.05

NOTE. Values expressed as OR (95% CI). Model 1, adjusted for age; model 2, adjusted for age, systolic blood pressure, antihypertensive medication, hemoglobin A_{1c} level, total cholesterol level, HDL cholesterol level, serum albumin level, habitual smokers, regular alcohol intake, and proteinuria; model 3, adjusted for confounding factors used in model 2 and inverse serum creatinine. To convert tHcy in μmol/L to mg/L, divide by 7.397.

*$P < 0.05$ versus low tertile.

levels were independently associated with microalbuminuria,[9,40] a marker of endothelial dysfunction and a predictor of future cardiovascular disease and renal dysfunction.[41-44] It is well recognized that there are histological and immunohistochemical similarities between the evolving fatty streak in the atherosclerotic vessel wall and progressive glomerular lesions leading to glomerulosclerosis.[45,46] These findings may give additional support to the hypothesis that tHcy leads to glomerulosclerosis by inducing vascular damage.

In our study, the frequency of regular alcohol intake decreased with increasing tHcy levels. Although chronic alcoholism clearly increases serum tHcy levels, possibly because of vitamin deficiencies,[47,48] effects of mild to moderate alcohol consumption are debated in the epidemiological studies.[49,50] In our subjects, alcohol consumption was associated positively with serum tHcy level ($r = 0.19$; $P < 0.01$). The majority of our subjects who consumed alcoholic beverages were mild and moderate alcohol drinkers. Thus, our findings suggest that mild to moderate alcohol intake exerts a favorable influence on tHcy levels.

Several limitations of our study should be discussed. The primary limitation is that our results might be biased by the exclusion of 111 subjects for whom serum tHcy levels could not be determined because of the lack of a serum sample or inadequate serum samples. These subjects might have had high serum tHcy levels because their mean age and systolic blood pressure levels were higher than those of the subjects in the present study. Additionally, it is possible that our results are biased by the exclusion of subjects who did not return for the follow-up examination. Among 824 subjects without a follow-up examination (431 men, 393 women), 103 patients died during the follow-up period: 45 of these patients died of cancer; 23 patients, cardiovascular disease; and the remaining subjects, other diseases. At baseline, mean age for men was significantly older in subjects with a follow-up examination than in those without it (58 versus 56 years), but this trend did not hold for women. However, mean values for tHcy, GFR, systolic and diastolic blood pressure, and frequency of proteinuria were not significantly different between the 2 groups for both sexes. Thus, this bias has the potential to alter our findings, but is not likely to do so.

A second limitation is that our GFR estimates made using the simplified prediction equation derived from the MDRD study and based on a single blood sample might not be sufficiently correct, although this prediction equation, among other equations of its type, is considered to be the most precise estimate of GFR.[30] In addition, a recent report showed that repeated serum creatinine measurements were necessary to correct within-person measurement variations in serum creatinine level (1% to ~10%),[51] suggesting that some nondifferential misclassifications of cases with CKD may have occurred in our study. Given that these limitations can reduce the impact of tHcy, the true association may be stronger than that shown in our findings.

A third limitation is that we have no data for vitamin status, including the status of folic acid, vitamin B_6, and vitamin B_{12}, or methylenetetrahydrofolate reductase (MTHFR) polymorphism, which affects serum tHcy levels.[10,52] Sarnak et al[27] reported that lower serum folate, pyridoxal 5-phosphate (active form of vitamin B_6), and vitamin B_{12} levels were not associated with the decline in GFR in multivariable analysis. To our knowledge, no previous study evaluated the relationship between MTHFR polymorphism and progression of kidney disease. Thus, it is not clear that vitamin status and MTHFR polymorphism influence the association between tHcy level and CKD. Additional examination on this issue is needed.

A fourth limitation is that tHcy concentrations were determined by using serum samples that had been frozen for 12 years. In theory, use of frozen samples can result in incorrect values because of either sample breakdown or hemoconcentration caused by dehydration of the sample. However, several studies have documented the stability of tHcy samples frozen for as long as 10 years,[53,54] and other studies also used frozen samples to assay tHcy.[16,17,52] Additionally, distributions of serum tHcy levels in our population were similar to those in other Japanese populations.[38] Thus, this limitation seems not to invalidate the association of tHcy level with the development of CKD found in our subjects.

The final limitation is that we have no information about type of underlying renal disease. Such

information could be obtained by detailed clinical examination, including renal biopsy and ultrasonography, but these diagnostic procedures are not considered feasible for a cohort study recruited from a general population, such as ours.

In conclusion, the findings of this study suggest that elevated tHcy levels are a significant risk factor for the development of CKD in the general population. At present, the extent to which tHcy-lowering treatment can attenuate the risk for CKD is not known. Thus, a tHcy-lowering clinical trial is needed to clarify whether the reduction in tHcy concentrations will result in an improved renal prognosis.

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RAPID COMMUNICATION
Clinical

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J Dent Res 83(6):485-490, 2004

The Severity of Periodontal Disease is Associated with the Development of Glucose Intolerance in Non-diabetics: The Hisayama Study

ABSTRACT

Inflammation is hypothesized to play a significant role in the development of type 2 diabetes; however, reports on clinical inflammatory conditions are limited. Studies have suggested that periodontitis affects glucose control in diabetics. This community-based study examined the relationship between periodontitis and glucose tolerance status, including changes in status. The relationship between periodontal condition and the results of a 75-g oral glucose tolerance test was examined in 961 adults in 1998. Deep pockets (mean pocket depth > 2.0 mm) were significantly associated with impaired glucose tolerance and with diabetes as compared with shallow pockets (< 1.3 mm). In the subgroup with normal glucose tolerance 10 years previously, subjects who subsequently developed impaired glucose tolerance were significantly more likely to have deep pockets. Deep pockets were closely related to current glucose tolerance status and the development of glucose intolerance.

KEY WORDS: periodontal disease, diabetes, glucose tolerance, risk factor, epidemiology.

Received May 21, 2003; Last revision March 28, 2004; Accepted April 9, 2004

INTRODUCTION

Recent evidence suggests that chronic subclinical inflammation plays an intermediary role in the pathogenesis of type 2 diabetes (Festa *et al.*, 2000; Pradhan *et al.*, 2001). Elevated levels of inflammatory markers, such as C-reactive protein (CRP) and interleukin-6 (IL-6), are reported to be significant risk indicators of type 2 diabetes (Pradhan *et al.*, 2001). However, few studies have examined the clinical conditions that cause chronic inflammation. Periodontal disease is a very common chronic subclinical inflammation, which the majority of affected people do not notice, even if they have been affected for years. In the US, about 30% of adults have periodontal disease with periodontal pockets ≥ 4 mm deep, which are a hotbed of subgingival anaerobes, such as *Porphyromonas gingivalis* (Brown *et al.*, 1996; Albandar *et al.*, 1999). Many studies have long recognized that periodontitis is more prevalent in diabetic patients and worsens with diabetes (Page *et al.*, 1997). Moreover, studies have indicated that treating periodontitis in diabetic patients has a beneficial effect on their glucose control (Williams and Mahan, 1960; Miller *et al.*, 1992; Grossi *et al.*, 1997; Collin *et al.*, 1998; Grossi, 2001; Iwamoto *et al.*, 2001; Stewart *et al.*, 2001). A randomized clinical trial in Pima Indians with type 2 diabetes demonstrated that periodontal treatment with doxycycline reduced *P. gingivalis* in periodontal pockets and also reduced hemoglobin A1c (HbA1c) after 3 mos (Grossi *et al.*, 1997; Grossi, 2001). The HbA1c level deteriorated in type 2 diabetic patients with, but not in patients without, severe periodontitis (Collin *et al.*, 1998). Elevated serum CRP (Wakai *et al.*, 1999; Loos *et al.*, 2000; Slade *et al.*, 2000; Wu *et al.*, 2000; Noack *et al.*, 2001; Glurich *et al.*, 2002; Saito *et al.*, 2003) and IL-6 (Loos *et al.*, 2000) levels have been reported in subjects with periodontitis. A recent study reported that serum IgG titers against *P. gingivalis* were correlated with CRP in Japanese type 2 diabetic subjects (Nishimura *et al.*, 2002). Therefore, we hypothesized that periodontal disease is a risk factor for type 2 diabetes. However, no studies have examined the relationship between periodontal disease and longitudinal changes in glucose control in non-diabetic subjects. In 1998, we conducted a periodontal examination as part of the Hisayama Study (Kubo *et al.*, 1999). In this article, we examine the relationship between periodontal condition and glucose tolerance using a cross-sectional, retrospective cohort design that considers past glucose tolerance.

MATERIALS & METHODS

The Hisayama Study began in 1961 and is an ongoing prospective cohort study of cardiovascular diseases. As part of the survey, between July and September, 1998, a total of 2180 Hisayama residents aged 40 to 79 yrs (52.1% of the total population in that age group) underwent a comprehensive health examination that included a fasting 75-g oral glucose tolerance test (OGTT). In all, 1111 of these residents underwent a periodontal examination. We excluded the 150

Table 1. Comparison of Subjects' Characteristics According to Glucose Tolerance Status in 1998 (n = 961)

Characteristics	Normal Glucose Tolerance (NGT, n = 669, 69.6%)	Impaired Glucose Tolerance (IGT, n = 191, 19.9%)	Diabetes (n = 101, 10.5%)	P[a]
Periodontal Condition		*Mean ± SD*		
Mean pocket depth (mm)	1.6 ± 0.5	1.7 ± 0.5[c]	1.8 ± 0.6[d]	
Maximum pocket depth (mm)	3.5 ± 1.4	3.9 ± 1.5[b]	4.0 ± 1.7[c]	
Mean attachment loss (mm)	1.9 ± 0.8	2.0 ± 0.9	2.3 ± 0.9[de]	
Maximum attachment loss (mm)	4.1 ± 1.71	4.5 ± 2.1	4.9 ± 2.1[c]	
Number of teeth	25.4 ± 4.0	24.9 ± 3.9	23.6 ± 4.0[de]	
Dental plaque index	1.0 ± 0.6	1.2 ± 0.7[c]	1.3 ± 0.7[d]	
Mean pocket depth (mm)		*No. of subjects (%)*		
Low (< 1.3)	230 (78.0)	47 (15.9)	18 (6.1)	0.0001
Intermediate (1.3-2.0)	322 (69.0)	94 (20.1)	51 (10.9)	
High (> 2.0)	117 (58.8)	50 (25.1)	32 (16.1)	
Mean attachment loss (mm)				
Low (< 1.5)	184 (71.0)	57 (22.0)	18 (6.9)	< 0.0001
Intermediate (1.5-2.5)	366 (73.1)	90 (18.0)	45 (9.0)	
High (> 2.5)	119 (59.2)	44 (21.9)	38 (18.9)	
General condition		*Mean ± SD*		
Age (yrs)	55.6 ± 8.8	57.0 ± 8.9	60.5 ± 6.9[df]	
BMI (kg/m^2)	22.8 ± 2.9	24.2 ± 3.6[d]	24.4 ± 3.6[d]	
HbA1c (%)	5.0 ± 0.2	5.2 ± 0.4[d]	6.4 ± 1.1[df]	
Triglyceride (mg/dL)	120 ± 90	136 ± 78	164 ± 94[de]	
Total cholesterol (mg/dL)	205 ± 35	208 ± 37	215 ± 38[b]	
HDL cholesterol (mg/dL)	59 ± 14	55 ± 13[c]	55 ± 14[b]	
LDL cholesterol (mg/dL)	122 ± 32	126 ± 33	128 ± 38	
Systolic blood pressure (mm Hg)	126 ± 20	133 ± 18[d]	142 ± 22[df]	
Diastolic blood pressure (mm Hg)	77 ± 11	80 ± 10[c]	82 ± 12[d]	
Smoking habits		*No. of subjects (%)*		
Never	466 (72.5)	121 (18.8)	56 (8.7)	0.049
Past	89 (62.7)	32 (22.5)	21 (14.8)	
Current	114 (64.8)	38 (21.6)	24 (13.6)	
Alcohol consumption (g/month)				
0	408 (72.2)	109 (19.3)	48 (8.5)	0.059
1-399	116 (69.9)	30 (18.1)	20 (12.0)	
400-1199	87 (65.4)	31 (23.3)	15 (11.3)	
≥ 1200	58 (59.8)	21 (21.6)	18 (18.6)	
Exercise frequency (times/wk)				
0	495 (70.2)	143 (20.3)	67 (9.5)	0.20
1-2	90 (73.2)	19 (15.4)	14 (11.4)	
≥ 3	84 (63.2)	29 (21.8)	20 (15.0)	
Sex				
Men	233 (61.8)	83 (22.0)	61 (16.2)	< 0.0001
Women	436 (74.7)	108 (18.5)	40 (6.8)	

[a] Actual probability by Pearson's chi-square test to compare the proportion of subjects.
[b-f] Student's *t* test or Welch's *t* test in case of unequal variances was applied with Bonferroni's correction of p-values for type 1 errors in multiple comparisons.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$, compared with NGT.
[e] $p < 0.05$.
[f] $p < 0.001$, comparison between diabetes and IGT.

subjects with fewer than 10 teeth from this study, because of the inherent difficulties in properly assessing periodontal health in these patients. Ultimately, 961 subjects (377 men and 584 women) with at least 10 teeth were analyzed.

In 1988, 2480 Hisayama residents aged 40 to 79 yrs underwent a similar health examination without a periodontal examination (Kubo *et al.*, 1999). Of the 961 subjects examined in 1998, 244 subjects under 50 yrs of age were excluded, since they were under 40 yrs of age in 1988. The 591 subjects for whom OGTT results in 1988 were available (82.4%) were enrolled in this study. Of these, 415 subjects had normal glucose tolerance in 1988 (152 men and 263 women, 50-79 yrs old in 1998), and we analyzed the relationship between periodontal conditions and the development of glucose intolerance between 1988 and 1998.

In the 1998 examination, following the method of the Third National Health and Nutrition Examination Survey (NHANES III) (Brown *et al.*, 1996), a periodontal examination was performed on 2 randomly selected quadrants, 1 maxillary and 1 mandibular, by four trained dentists, using a normal dental chair. Mean periodontal pocket depth and attachment loss were analyzed. The subjects were divided into 3 categories with respect to each of the 2 periodontal measurements, mean pocket depth and mean attachment loss: 'Low' (< 1.3 mm), 'Intermediate' (1.3-2.0 mm), and 'High' (> 2.0 mm) mean pocket depth; and 'Low' (< 1.5 mm), 'Intermediate' (1.5-2.5 mm), and 'High' (> 2.5 mm) mean attachment loss. For both measurements, the 'High' categories corresponded to the highest 20% of the measurements and the 'Low' to the lowest 30%. A report on NHANES III (Albandar *et al.*, 1999) showed that about 20% of

subjects 50-60 yrs old had moderate to advanced periodontitis. Accordingly, we used the top 20% for poor periodontal health. Subjects with means in the 30th percentile were assigned to the periodontally healthy group and the remaining 50% of the patients to the intermediate group.

The morning after subjects had fasted overnight, blood samples were collected from the antecubital vein and were analyzed according to previously described methods (Kubo *et al.*, 1999). The World Health Organization criteria for the diagnosis of diabetes were applied (Alberti and Zimmet, 1998): normal glucose tolerance (NGT, fasting and two-hour post-challenge plasma glucose levels < 110 mg/dL and < 140 mg/dL, respectively), diabetes (fasting or two-hour post-challenge plasma glucose levels ≥ 126 mg/dL or ≥ 200 mg/dL, respectively), and impaired glucose tolerance (IGT, all others with some glucose tolerance impairment including impaired fasting glucose, *i.e.*, with one of the two glucose tolerance levels between normal and diabetic values and the other below the diabetic level).

Glucose intolerance was defined as developing in subjects who had NGT in 1988, but had IGT or were diabetic in 1998. For 597 subjects, HbA1c data for both 1988 and 1998 were available. The change in HbA1c was defined as the 1998 value minus the 1988 value. An increase in HbA1c of ≥ 0.2%, which corresponded to the proportion of subjects in whom there was deterioration in OGTT, was considered progression. Progression (yes/no) in HbA1c served as the dependent variable in the logistic regression analysis. Each subject completed a self-administered questionnaire, which was checked by trained nurses. Age (continuous), sex, BMI (continuous), exercise frequency (0, 1-2, ≥ 3 times a wk), alcohol consumption (converted to 100% ethanol *per* month: 0, 1-399, 400-1199, ≥ 1200 g), and smoking habits (never, past, current smoker) were used as independent variables, all having been reported risk factors for type 2 diabetes in multivariate analyses (Hu *et al.*, 2001).

The differences in the mean values were evaluated by Student's *t* test and Welch's *t* test in the case of unequal variances. To protect against spurious significance among multiple inferences, we used Bonferroni's correction to interpret the significance of the *p*-values.**(AQ)** Differences in percentages were evaluated by Pearson's correlation, and trends were evaluated by the Mantel-Haenszel chi-squared test. We performed multivariate logistic regression analyses to determine the effect of periodontal condition on the glucose tolerance status, and calculated the odds ratio (OR) and 95% confidence interval (CI). SPSS version 11.0 (SPSS Japan Inc., Tokyo, Japan) was used for the analyses. The design of the study and procedures for obtaining informed consent were approved by the Ethics Committee of Kyushu University Faculty of Dental Science and the Department of Health and Welfare of Hisayama town.

RESULTS

In 1998, 191 of 961 subjects had IGT, and 101 had diabetes, based on the results of the OGTT. The characteristics of subjects with NGT, IGT, and diabetes were compared (Table 1). Periodontal condition and many of the other values were worse in subjects with poorer OGTT results. The numbers of subjects with NGT, IGT, and diabetes were compared according to periodontal condition by mean pocket depth and mean attachment loss (Table 1). The proportion of subjects with IGT and that with diabetes increased significantly with mean pocket depth ($P = 0.0001$). The proportion with diabetes increased with mean attachment loss, while the proportion with IGT did not. The increased proportion of subjects with IGT and with diabetes, according to mean pocket depth, was recognized in the univariate and multivariate analyses, as shown in Table 2. In the highest category of pocket depth, the adjusted OR for IGT to NGT was 1.8 (95% CI, 1.1-2.9) as compared with the lowest category of pocket depth, adjusted for age, sex, BMI, exercise frequency, alcohol consumption, and smoking habits (Table 2). The adjusted OR for diabetes in the intermediate and high categories of pocket depth was 1.9 (95% CI, 1.0-3.4) and 2.6 (95% CI, 1.3-5.0), respectively. While there was a significant relationship between diabetes and mean attachment loss in both the univariate and multivariate analyses, we could not find any significant relationship between IGT and mean attachment loss (Table 2).

The OGTT results were analyzed retrospectively for 591 subjects (Table 3). Of these, 415 subjects (70.2%) had NGT and 176 subjects (29.8%) had IGT or diabetes in 1988, and the

Table 2. Relationship between Periodontal Condition and Glucose Tolerance Status in 1998

	Subjects with NGT and with IGT, n = 860						Subjects with NGT and with Diabetes, n = 770					
			Univariate Analysis		Multivariate Analysis[a]				Univariate Analysis		Multivariate Analysis[a]	
Periodontal Condition	NGT	IGT	P[b]	P[c]	OR (95% CI)	P	NGT	Diabetes	P[b]	P[c]	OR (95% CI)	P
Mean pocket depth (mm)	No. of subjects (%)		0.006	0.001			No. of subjects (%)		0.0002	< 0.0001		
Low (< 1.3)	230 (83.0)	47 (17.0)			1		230 (92.7)	18 (7.3)			1	
Intermediate (1.3-2.0)	322 (77.4)	94 (22.6)			1.4 (0.9-2.0)	0.13	322 (86.3)	51 (13.7)			1.9 (1.0-3.4)	0.037
High (> 2.0)	117 (70.1)	50 (29.9)			1.8 (1.1-2.9)	0.013	117 (78.5)	32 (21.5)			2.6 (1.3-5.0)	0.004
Mean attachment loss (mm)			0.13	0.60					< 0.0001	< 0.0001		
Low (< 1.5)	184 (76.3)	57 (23.7)			1		184 (91.1)	18 (8.9)			1	
Intermediate (1.5-2.5)	366 (80.3)	90 (19.7)			0.8 (0.5-1.1)	0.16	366 (89.1)	45 (10.9)			1.1 (0.6-2.0)	0.83
High (> 2.5)	119 (73.0)	44 (27.0)			1.0 (0.6-1.7)	0.97	119 (75.8)	38 (24.2)			2.0 (1.0-3.9)	0.038

[a] Logistic regression analysis adjusted for age (continuous), sex, BMI (continuous), exercise frequency (0, 1-2, ≥ 3 times a wk), alcohol consumption (converted to 100% ethanol *per* month; 0, 1-399, 400-1199, ≥ 1200 g), and smoking habits (never, past, current smoker).
[b] P value for the non-linear component calculated by Pearson's chi-square test.
[c] P value for the linear component calculated by the Mantel-Haenszel chi-square test.

Table 3. Relationship between Periodontal Condition in 1998 and Glucose Tolerance Status in 1988 and 1998

	All Subjects in 1988, n = 591				All Subjects in 1988, n = 591				Limited to Subjects with NGT in 1988, n = 415			
	OGTT in 1988				OGTT in 1998				OGTT in 1998			
Periodontal Condition	NGT	IGT and Diabetes	P[a]	P[b]	NGT	IGT and Diabetes	P[a]	P[b]	NGT	IGT and Diabetes	P[a]	P[b]
Mean pocket depth (mm)	No. of subjects (%)		0.039	0.016	No. of subjects (%)		0.0001 < 0.0001		No. of subjects (%)		0.003	0.0007
Low (< 1.3)	123 (77.8)	35 (22.2)			126 (79.7)	32 (20.3)			110 (89.4)	13 (10.6)		
Intermediate (1.3-2.0)	202 (68.5)	93 (31.5)			193 (65.4)	102 (34.6)			160 (79.2)	42 (20.8)		
High (> 2.0)	90 (65.2)	48 (34.8)			79 (57.2)	59 (42.8)			64 (71.1)	26 (28.9)		
Mean attachment loss (mm)			0.12	0.038			0.11	0.053			0.23	0.16
Low (< 1.5)	97 (75.8)	31 (24.2)			91 (71.1)	37 (28.9)			80 (82.5)	17 (17.5)		
Intermediate (1.5-2.5)	220 (70.7)	91 (29.3)			215 (69.1)	96 (30.9)			181 (82.3)	39 (17.7)		
High (> 2.5)	98 (64.5)	54 (35.5)			92 (60.5)	60 (39.5)			73 (74.5)	25 (25.5)		
Total	415 (70.2)	176 (29.8)			398 (67.3)	193 (32.7)			334 (80.5)	81 (19.5)		

[a] P value for the non-linear component was calculated by Pearson's chi-square test.
[b] P value for the linear component was calculated by the Mantel-Haenszel chi-square test.

proportions with IGT and diabetes increased slightly in 1998 (32.7%). The proportion with IGT and diabetes in 1988 (Table 3, left column) was greater in deeper pocket depth categories (P for trend = 0.016). The relationship between pocket depth and the proportion with IGT and diabetes was stronger in 1998 (P for trend < 0.0001) than in 1988 (Table 3).

Of the 415 subjects with NGT in 1988, 81 subjects (19.5%) had IGT or diabetes in 1998. The proportion with IGT and of diabetic subjects who had NGT in 1988 increased with mean pocket depth (P for trend = 0.0007; Table 3, right column), more than was the case for all of the subjects (center column). Of the 176 subjects with IGT or diabetes in 1988, there were 64 subjects (36.4%) whose glucose tolerance condition had converted to NGT (data not shown). The proportion of these subjects was larger in subjects with a lower mean pocket depth than in those with deeper pockets, but the trend did not reach statistical significance (P = 0.19). The relationship between attachment loss and OGTT results in 1998 was not significant.

Table 4 compares the proportions with IGT with those with NGT and those with diabetes with those with NGT separately for the same 415 subjects with NGT in 1988. The proportion of subjects with IGT increased more in those with deeper pockets (P for trend = 0.001). The multivariate logistic regression analysis, with the deterioration in glucose tolerance over the 10-year interval as the dependent variable (yes/no), shows that the intermediate and high categories of mean pocket depth were significantly associated with a deterioration in the OGTT from NGT to IGT, as compared with the deterioration of those in the lowest category of mean pocket depth (Table 4). The OR was greater in subjects with deeper periodontal pockets (P for trend = 0.018 in the multivariate analysis). Mean attachment loss was analyzed in the same manner, but no significant association was observed (Table 4). The OR for diabetes also increased with pocket depth, but did not reach statistical significance (Table 4) due to the small sample size and corresponding lack of power.

Of the 597 subjects for whom HbA1c data were available in both 1988 and 1998, each additional millimeter in mean pocket depth corresponded to a 0.13% increase in HbA1c, according to a multivariate linear regression (P = 0.007). Excluding the 52 subjects diagnosed with diabetes in 1988, the subjects in the high category of pocket depth had a significant increase in HbA1c of $\geq$ 0.2% from 1988 to 1998 (18% of the 545 non-diabetic subjects), as compared with subjects in the low category, according to a similar multivariate analysis (OR, 2.4; 95% CI, 1.2-4.6; P = 0.009). The mean attachment loss was not associated with an increase in HbA1c according to a similar analysis.

DISCUSSION

In a cross-sectional analysis of the 1998 data, deep periodontal pockets were significantly associated with IGT and diabetes, whether in univariate or multivariate models controlling for known risk factors for diabetes. Since worsening of the diabetic condition is associated with deteriorating periodontal tissue (Page *et al.*, 1997), a cross-sectional relationship between deep pockets and diabetes was presumed. Although no increased risk of periodontal disease with IGT has been reported, our results suggest the hypothesis that not only diabetes but also IGT increases the risk of deeper pockets. Considering the possible effects of diabetic and IGT conditions on periodontal tissue, we excluded subjects with diabetes and with IGT in 1988 retrospectively. As expected, the OR for periodontal condition and the development of glucose intolerance from NGT in 1988 to IGT in 1998 (Table 4) was higher and the p-value lower than in the cross-sectional analysis (Table 2), although there were fewer subjects. Moreover, the relationship between pocket depth and glucose intolerance in 1998 was stronger than the relationship between pocket depth and glucose intolerance in 1988 (Table 3). These results indicate that deep pockets were more closely associated with the development of glucose intolerance from normal status than the past glucose tolerance status itself. About one-third of the subjects with IGT or diabetes in 1988 improved their glucose status to NGT in 1998. In this subgroup, the proportion with NGT in 1998 was higher

Table 4. Relationship between Periodontal Condition and Glucose Tolerance Status in 1998, Limited to the Subjects with Normal Glucose Tolerance in 1988

	Subjects with NGT and with IGT in 1998, n = 406						Subjects with NGT and with Diabetes in 1998, n = 343					
			Univariate Analysis		Multivariate Analysis[a]				Univariate Analysis		Multivariate Analysis[a]	
Periodontal Condition	NGT	IGT	P[b]	P[c]	OR (95% CI)	P	NGT	Diabetes	P[b]	P[c]	OR (95% CI)	P
Mean pocket depth (mm)	*No. of subjects (%)*		0.005	0.001			*No. of subjects (%)*		0.37	0.30		
Low (< 1.3)	110 (90.2)	12 (9.8)			1		110 (99.1)	1 (0.9)			1	
Intermediate (1.3-2.0)	160 (81.6)	36 (18.4)			2.1 (1.0-4.2)	0.048	160 (96.4)	6 (3.6)			5.0 (0.6-45)	0.15
High (> 2.0)	64 (72.7)	24 (27.3)			3.1 (1.4-6.9)	0.005	64 (97.0)	2 (3.0)			3.7 (0.3-47)	0.31
Mean attachment loss (mm)			0.28	0.24					0.57	0.29		
Low (< 1.5)	80 (83.3)	16 (16.7)			1		80 (98.8)	1 (1.2)			1	
Intermediate (1.5-2.5)	181 (84.2)	34 (15.8)			0.9 (0.4-1.7)	0.66	181 (97.3)	5 (2.7)			3.6 (0.4-34)	0.27
High (> 2.5)	73 (76.8)	22 (23.2)			1.3 (0.6-2.9)	0.49	73 (96.1)	3 (3.9)			4.8 (0.4-55)	0.21

[a] Logistic regression analysis adjusted for age (continuous), sex, BMI (continuous), exercise frequency (0, 1-2, ≥ 3 times a wk), alcohol consumption (converted to 100% ethanol *per* month; 0, 1-399, 400-1199, ≥ 1200 g), and smoking habits (never, past, current smoker).
[b] P value for the non-linear component calculated by Pearson's chi-square test.
[c] P value for the linear component calculated by the Mantel-Haenszel chi-square test.

in subjects with shallower pocket depths than in those with deeper pocket depths, although it did not reach statistical significance (data not shown). This may relate to previous reports that periodontal treatment has beneficial effects on glucose control in diabetics.

The OR for diabetes from NGT in the past 10 years was not significant, since there were only nine diabetic subjects (Table 4). The analyses of HbA1c over the 10-year period showed that it increased more in subjects with deep periodontal pockets, supporting the results of the OGTT. In all the analyses, severe attachment loss was not associated with IGT, although it was significantly associated with diabetes in cross-sectional analyses, as is well-known (Page *et al.*, 1997). Since attachment loss usually means gingival recession plus periodontal pockets, such patients are less likely to harbor subgingival bacteria than those with deep periodontal pockets. Generally, periodontal pockets are directly related to subgingival bacteria, while attachment loss is not. From these results, especially from the retrospective analyses, chronic inflammation from subgingival pathogens in deep periodontal pockets may affect glucose control in non-diabetic subjects.

Tumor necrosis factor alpha (TNF-α), which is produced from the increased amounts of adipose tissue in obese subjects, plays a predominant role in insulin resistance (Uysal *et al.*, 1997; Zinman *et al.*, 1999). In the periodontium, it is well-known that the lipopolysaccharides continuously provided by Gram-negative bacteria, such as *P. gingivalis*, trigger the production of TNF-α, a pro-inflammatory cytokine (Page *et al.*, 1997). The area of the interface where these subgingival bacteria can interact with gingival tissue is estimated to be as much as 72 cm^2 in patients with severe periodontitis and many deep pockets; this results in an enormous burden of Gram-negative bacteria (Page *et al.*, 1997). Periodontal treatment to remove these bacteria appears to reduce circulating TNF-α levels (Iwamoto *et al.*, 2001). TNF-α induced from periodontal pathogens may increase insulin resistance, which may lead to a risk of cardiovascular disease (Genco *et al.*, 2002).

There are several potential limitations to our findings. The participation rates by residents in the health and periodontal examinations were 52% and 26%, respectively. Subjects with fewer than 10 teeth were excluded. We had no data on subjects' periodontal condition in 1988. According to NHANES III, mean pocket depth was about the same in every age group, and the percentage of sites with deep pockets was the same in subjects over 50 yrs old, while attachment loss increased with age (Brown *et al.*, 1996; Albandar *et al.*, 1999). Indeed, in our subjects, the correlation coefficient between mean pocket depth and age was smaller ($r = 0.13$) than that between mean attachment loss and age ($r = 0.28$). These observations support the assumption that the periodontal pockets of the subjects examined in 1998 might not have changed much in the previous 10 years. Based on this assumption, we considered the possibility that periodontal disease had an adverse effect on glucose tolerance. Owing to its cross-sectional character, based on a 1998 examination with additional 1988 OGTT data, the study cannot provide a clear answer to the question of whether having deep pockets is the cause or the result of IGT. We can conclude that deep pockets and current glucose tolerance status, such as IGT and diabetes, are significantly associated. The significant relationship between deep pockets and the past development of glucose intolerance in non-diabetics suggests that periodontal disease is a risk factor for type 2 diabetes. Prospective cohort studies with sufficient subjects are required to confirm this suggestion.

ACKNOWLEDGMENTS

We thank Daisuke Ikeda, DDS, and Atsushi Hideshima, DDS, for periodontal examination. This work was supported by Grants-in-Aid of Scientific Research (No. 15390652 and 13670370) from the Ministry of Education, Science, Sports and Culture of Japan. This article is respectfully dedicated to the memory of Toshihiko Koga, who died on 14 October 2001.

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Relationship Between Electrocardiographic Abnormalities and Periodontal Disease: The Hisayama Study

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Background: Recent studies have suggested a relationship between periodontitis and cardiovascular disease (CVD). This study investigated the relationship between periodontitis and electrocardiographic (ECG) abnormalities, which are known predictors of CVD.

Methods: We examined the periodontal status of 1,111 residents of Hisayama Town, Fukuoka, Japan. Nine hundred fifty-seven (957) subjects (374 males, 583 females) with ≥10 teeth and without a medical history of CVD were included in the analysis. Probing depth (PD) and clinical attachment level (CAL) were measured on two randomly selected quadrants, one maxillary and one mandibular. A 12-lead ECG was recorded using a standard electrocardiograph. ECG abnormalities included left ventricular hypertrophy (Minnesota code 3-1) and ST depression (4-1, 2, 3). The relation of periodontal condition and ECG abnormalities was assessed with logistic regression analysis.

Results: Univariate analysis revealed that mean probing depth, mean attachment loss, number of teeth, and plaque index were significantly associated with ECG abnormalities, as well as with known risk factors of CVD. In multivariate analysis, the subjects with deep pockets (mean probing depth ≥2 mm) had an increased risk for ECG abnormalities (odds ratio [OR] = 1.6; 95% confidence interval [CI] = 1.01 to 2.50) compared to the subjects with mean PD <2 mm. Subjects with severe attachment loss (mean CAL ≥2.5 mm) had also significant risk for ECG abnormalities (OR = 1.7; 95% CI = 1.07 to 2.67) compared to those whose mean CAL was <2.5 mm.

Conclusion: This study clearly shows the relationship between periodontitis and ECG abnormalities, which are important predictors of CVD. *J Periodontol 2004;75:791-797.*

KEY WORDS

Coronary disease/epidemiology; electrocardiography/abnormalities; periodontitis/complications; risk factors.

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Accumulated epidemiologic and laboratory evidence suggests that periodontal infection is a contributing risk factor for cardiovascular disease (CVD), a serious disease often leading to death.[1-6] Inflammation due to infection by several microorganisms, such as *Chlamydia pneumoniae, Helicobacter pylori*, and cytomegalovirus, has been implicated in the etiology of atherosclerosis, which is intrinsic to CVD.[7,8] Periodontitis is a chronic inflammatory disease caused by Gram-negative anaerobic bacteria, such as *Porphyromonas gingivalis* and *Tannerella forsythensis*, and is most prevalent in adults. Periodontal pathogens exist in atherosclerotic plaques, where they may play a role in the development and progression of atherosclerosis, suggesting a direct influence of periodontal pathogens on CVD.[9,10] However, some studies show no or at most a weak relationship between periodontitis and CVD.[11-14] Therefore, further evidence is required to clarify this relationship.[15]

Electrocardiographic (ECG) abnormalities are significantly related to subsequent death from coronary heart disease (CHD),[16] and are one of the most sensitive predictors of fatal CHD.[17] In particular, left ventricular hypertrophy (LVH) and ST depression are thought to be important predictors of heart disease and death from coronary events.[18-25] As ECG examinations take only a few minutes, cause no discomfort, and do not require the presence of a physician, they are widely used to screen for heart disease

in health examinations in Japan. An examination of the relationship between periodontitis and ECG abnormalities may produce detailed information on the effect of periodontitis on conditions preexisting CVD. This study examined the relationship between periodontal condition and ECG abnormalities in subjects without a medical history of CVD who underwent a health examination as part of the Hisayama Study.[26]

MATERIALS AND METHODS

Study Population

Hisayama is a subrural town that is adjacent to Fukuoka City, on Kyushu Island, in southern Japan. The population of the town has been approximately 7,000 for the past 30 years. Between July and September 1998, 2,180 residents (adult population between 20 and 81 years of age, mean age 55.8 ± 9.6 convenience sample) underwent a comprehensive health examination. We examined only the 1,111 dentate subjects, who received both oral and general examinations. The remaining 1,069 adults were either edentulous or refused an oral examination. Exclusion criteria included individuals with <10 teeth (because of the inherent difficulties in properly assessing periodontal health) and those with a medical history of CVD. Consequently, 957 individuals (374 males, 583 females) with ≥10 teeth and without a medical history of CVD were included. The design of the study, its data collection methods, and the procedures used to obtain informed consent were approved by the Department of General Affairs and Health and Welfare of Hisayama.

Oral Examination

The periodontal examination was performed on two randomly selected quadrants, one maxillary and one mandibular, following the method of the National Health and Nutrition Examination Survey III (NHANES III).[27] The periodontal examination was carried out by one of four dentists trained to perform a clinical examination of oral health status. The examiner reliability of the periodontal examination was verified by an interexaminer calibration of outpatients visiting Kyushu University Dental Hospital; the kappa value for the periodontal examination exceeded 0.8, suggesting very good inter-examiner agreement. Probing depth (PD) and clinical attachment level (CAL) were measured at mesio-buccal and mid-buccal sites for all of the teeth present in the two quadrants. Each subject's periodontal condition was classified according to their mean PD: ≥2 mm (20.1%) or <2 mm, and mean CAL: ≥2.5 mm (20.4%) or <2.5 mm. NHANES III found that about 20% of 50- to 60-year-old subjects had moderate to advanced periodontitis.[28] Accordingly, we used the 20th percentile to indicate poor periodontal conditions. Other oral examinations were performed following the methods recommended by the World Health Organization (WHO). Oral hygiene status was evaluated using a plaque index.[29]

General Examination

A 12-lead ECG was recorded in the supine position using a standard electrocardiograph. Using the Minnesota code[30] to evaluate the ECG, we defined LVH (Minnesota code 3-1) and ST-segment depression (4-1, 2, 3) as ECG abnormalities, both of which are considered important predictors of heart disease and death from coronary events.[18-25] Blood pressure was measured three consecutive times, after resting for at least 5 minutes, using a standard mercury sphygmomanometer with the subjects in the sitting position and the average value was used for the analysis. Blood samples were collected from an antecubital vein after an overnight fast. The laboratory analyses of the blood samples followed previously described methods.[26] The subjects were evaluated with a 75 g oral glucose tolerance test and categorized into three groups: normal (fasting and 2-hour post-challenged plasma glucose levels <110 and <140 mg/dl, respectively), diabetic (fasting or 2-hour post-challenged plasma glucose levels ≥126 or ≥200 mg/dl, respectively), and impaired (other than the above). The body-mass index (BMI) was defined as the weight in kilograms divided by the square of the height in meters. Each subject completed a self-administrated questionnaire in advance, which was checked by trained nurses. The questionnaire included social status, exercise frequency (0, 1-2, ≥3 times a week), alcohol consumption (converted to 100% ethanol per month; 0, 1-399, 400-1199, ≥1200 g), and smoking status (never, former, current smoker).

Statistical Analysis

The subjects' characteristics were compared statistically using the *t* test for quantitative data and the chi-square test for the ratio of categorical variables between subjects with and without ECG abnormalities. We recognized a significant relationship ($P<0.001$) between the periodontal parameters as an independent variable and ECG abnormalities as the dependent variable in a univariate logistic regression analysis. Subsequently, we added the variables that were significantly related to ECG abnormalities in the univariate analysis to a bivariate logistic regression analysis with the periodontal parameter and evaluated them individually for both confounding and statistical significance. The variables that showed the statistical significance in the bivariate analysis with the periodontal parameters were entered into a multivariate logistic regression analysis to calculate the odds ratio (OR) and 95% confidence interval (CI) for ECG abnormalities as the dependent variable. All analyses were performed using a software program.§

§ SPSS Version 6.1, SPSS Japan Inc., Tokyo, Japan.

RESULTS

Table 1 shows the relationship between ECG abnormalities and each study variable. The variables that were significantly associated with ECG abnormalities in the univariate analysis were age, number of teeth, systolic blood pressure, diastolic blood pressure, mean PD, mean CAL, gender, plaque index, smoking status, alcohol consumption, and exercise frequency. There were 147 subjects who had both a mean PD ≥2 mm and a mean CAL ≥2.5 mm (70.3% of the subjects who had a mean PD ≥2 mm and 67.7% of the subjects who had a mean CAL ≥2.5 mm). Since the mean PD and mean CAL, which represent the subjects' periodontal condition, were closely related ($r = 0.78$, $P < 0.001$), these two variables were analyzed separately in the following analyses. Variables that were significant in the univariate analysis were subjected to a bivariate logistic regression analysis with the periodontal parameters. Age, gender, number of teeth, systolic blood pressure, diastolic blood pressure, smoking status, alcohol consumption, and exercise frequency were the significant variables in the analysis when considered with each of the two periodontal parameters (mean PD and mean CAL). All of the significant variables and the periodontal parameters were then subjected to a multivariate logistic regression analysis. We used systolic blood pressure as the representative blood pressure. As a result, mean PD, mean CAL, age, and systolic blood pressure were identified as significant risk factors for ECG abnormalities (Tables 2 and 3). The results for mean PD and mean CAL were similar, and the OR for ECG abnormalities for the subjects whose mean PD was ≥2 mm was 1.6 compared to the subjects with

Table 1.

Population Characteristics of Subjects According to ECG Status

	ECG Abnormalities		
	Negative (N = 832)	Positive (N = 125)	
	Mean ± SD		*P* Value*
Age (years)	55.1 ± 9.6	60.2 ± 8.9	<0.001
Teeth (N)	25.4 ± 3.9	23.9 ± 4.2	<0.001
Decayed teeth (N)	0.7 ± 1.4	0.7 ± 1.5	NS
Body-mass index (kg/m^2)	23.2 ± 3.3	22.8 ± 3.0	NS
Systolic blood pressure (mm Hg)	126.6 ± 19.1	142.9 ± 24.7	<0.001
Diastolic blood pressure (mm Hg)	77.5 ± 10.8	82.7 ± 10.5	<0.001
Triglyceride (mg/dl)	124.5 ± 85.5	138.4 ± 97.3	NS
HDL cholesterol (mg/dl)	58.3 ± 13.7	56.0 ± 13.1	NS
LDL cholesterol (mg/dl)	123.2 ± 32.3	117.7 ± 35.9	NS
Mean PD (mm)	1.6 ± 0.5	1.8 ± 0.6	<0.001
Mean CAL (mm)	1.9 ± 0.8	2.2 ± 0.8	<0.001
	N (%)		*P* Value†
Gender			
Male	311 (83.2)	63 (16.8)	<0.01
Female	521 (89.4)	62 (10.6)	
Mean PD			
<2 mm	665 (88.9)	83 (11.1)	<0.01
≥2 mm	167 (79.9)	42 (20.1)	
Mean CAL			
<2.5 mm	662 (89.5)	78 (10.5)	<0.001
≥2.5 mm	170 (78.3)	47 (21.7)	
Plaque index			
Low	404 (90.2)	44 (9.8)	<0.01
High	428 (84.1)	81 (15.9)	
Social class			
Managerial position	50 (89.3)	6 (10.7)	NS
Office worker	446 (88.1)	60 (11.9)	
Primary industries	58 (76.3)	18 (23.7)	
Factory worker	77 (80.2)	19 (19.8)	
Housewife or without job	201 (90.1)	22 (9.9)	
Smoking status			
Never	568 (88.9)	71 (11.1)	<0.05
Former	111 (81.6)	25 (18.4)	
Current	153 (84.1)	29 (15.9)	
Alcohol consumption (converted to 100% ethanol per month)			
0-399 g	647 (88.6)	83 (11.4)	<0.01
400-1199 g	114 (85.1)	20 (14.9)	
≥1200 g	71 (76.3)	22 (23.7)	

Table 1. (continued)

Population Characteristics of Subjects According to ECG Status

	ECG abnormalities		
	Negative (N = 832)	Positive (N = 125)	*P* Value†
Exercise frequency			
0-2 times a week	595 (85.5)	101 (14.5)	<0.05
≥3 times a week	237 (90.8)	24 (9.2)	
Glucose tolerance‡			
Normal	587 (87.9)	81 (12.1)	NS
Impaired	159 (85.5)	27 (14.5)	
Diabetic	77 (82.8)	16 (17.2)	

* *t* test.
† Chi-square test.
‡ The 10 patients who underwent glucose testing were excluded.

Table 2.

A Multiple Logistic Regression Analysis of the Effect of the Explanatory Variables Including Mean PD on ECG Abnormalities

	Dependent Variable = ECG Abnormalities (negative = 0, positive = 1)				
Independent Variable	B	SE	*P* Value	OR	95% CI
Age	0.033	0.013	0.011	1.0	1.01 – 1.06
Gender (male = 0, female = 1)	–0.076	0.324	0.814	0.9	0.49 – 1.75
Mean PD (<2 mm = 0, ≥2 mm = 1)	0.463	0.231	0.045	1.6	1.01 – 2.50
N teeth	–0.023	0.026	0.383	1.0	0.93 – 1.03
Systolic blood pressure	0.028	0.005	<0.001	1.0	1.02 – 1.04
Smoking status					
Never				1.0	
Former	0.122	0.349	0.726	1.1	0.57 – 2.24
Current	0.205	0.330	0.534	1.2	0.64 – 2.35
Alcohol consumption					
0 – 399 g				1.0	
400 – 1199 g	0.133	0.320	0.678	1.1	0.61 – 2.14
≥1200 g	0.323	0.347	0.352	1.4	0.70 – 2.73
Exercise frequency (0-2 times a week = 0, ≥3 times a week = 1)	–0.439	0.254	0.084	0.6	0.39 – 1.06

a mean PD <2 mm (Table 2); the OR for the subjects with a mean CAL ≥2.5 mm was 1.7 compared with the subjects with a mean CAL <2.5 mm (Table 3). Older age and higher systolic blood pressure increased the risk of ECG abnormalities significantly. Exercise frequency did not reach statistical significance ($P = 0.084$, Table 2; $P = 0.069$, Table 3).

DISCUSSION

Resting ECG abnormalities were significant predictors of both total and fatal CHD.[17] Three Chicago epidemiological studies found that men with major ECG abnormalities had considerably higher death rates than those with a normal ECG, and in a multivariate analysis ECG abnormalities had a significant relationship to death from CHD and to death from all causes, independent of other confounding variables.[16] Therefore, ECG examinations are widely used to screen for heart disease in health examinations. We defined LVH and ST depression as ECG abnormalities using the Minnesota code, which has been used in many epidemiological studies. LVH suggesting hypertensive heart disease is one of the most frequent ECG findings in the asymptomatic general population in Japan and is considered a predictor of future atherosclerotic disease.[21,23] LVH was reported to be one of the predictors of sudden cardiac death among Hawaiian-Japanese men.[22] The Framingham study indicated that patients with LVH by ECG had a greater risk of sudden death and acute myocardial infarction than subjects with normal hearts.[19] In addition, ST depression suggesting ischemia has been reported to be an important predictor of CVD.[24,25] In healthy subjects, ST depressions in an ECG using the Minnesota code is associated with increased risk of coronary events.[18,20] De Bacquer et al. reported that both ST segment depression and LVH were the most predictive ECG findings for CVD death.[25] Considering these reports, the relationship between periodontitis and ECG abnormalities observed in this study suggests a relationship between periodontitis and CVD.

Table 3.

Multiple Logistic Regression Analysis of the Effect of the Explanatory Variables Including Mean CAL on ECG Abnormalities

	Dependent Variable = ECG Abnormalities (negative = 0, positive = 1)				
Independent Variable	B	SE	*P* Value	OR	95% CI
Age	0.030	0.013	0.021	1.0	1.00 – 1.06
Gender (male = 0, female = 1)	–0.110	0.324	0.735	0.9	0.47 – 1.69
Mean CAL (<2.5 mm = 0, ≥2.5 mm = 1)	0.523	0.234	0.025	1.7	1.07 – 2.67
N teeth	–0.019	0.026	0.470	1.0	0.93 – 1.03
Systolic blood pressure	0.028	0.005	<0.001	1.0	1.02 – 1.04
Smoking status					
Never				1.0	
Former	0.098	0.352	0.780	1.1	0.55 – 2.20
Current	0.138	0.337	0.682	1.1	0.59 – 2.22
Alcohol consumption					
0 – 399 g				1.0	
400 – 1199 g	0.145	0.321	0.653	1.2	0.62 – 2.17
≥1200 g	0.303	0.347	0.382	1.4	0.69 – 2.67
Exercise frequency (0-2 times a week = 0, ≥3 times a week = 1)	–0.464	0.255	0.069	0.6	0.38 – 1.04

Periodontal disease is a chronic inflammatory disease caused by Gram-negative anaerobic bacteria. Reports that periodontitis results in higher systemic levels of C-reactive protein (CRP), interleukin-6, and neutrophils suggest that elevated levels of these inflammatory substances cause inflammatory changes to atherosclerotic lesions, increasing the risk of cardiac or cerebrovascular events.[31,32] Periodontal pathogens, such as *Tannerella forsythensis, Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Prevotella intermedia*, and *Streptococcus sanguis*, have been detected in atherosclerotic plaques and implicated in the infectious etiology of atherosclerosis.[9,10] Herzberg and Meyer showed that *S. sanguis* contributed to acute thrombotic events in experimental rabbits;[33,34] *P. gingivalis* vesicles on the outer membrane have been reported to possess platelet aggregation activity.[35] These studies suggest potential causal mechanisms that may underlie the relationship between periodontitis and CVD.

Although several studies have reported no relationship between periodontitis and CVD,[11,12,14] these studies had limitations in the way they evaluated periodontal conditions. Howell et al. used a self-reported periodontal evaluation,[11] which is far from accurate compared with direct measurement using a periodontal probe. Genco et al. commented that the Russell periodontal index used in the National Health and Nutrition Examination Survey I[12] is subjective and less accurate than objective periodontal measurement and is thought to result in the misdiagnosis of periodontal disease.[36] In another study, Hujoel et al. defined edentulous status as completely eliminating dental infections, and showed that edentulous subjects did not have a lower CVD risk than subjects with periodontitis.[13] Both edentulous status and severe gingivitis are significant risks for CVD;[37] therefore, methods that compare edentulous status with periodontitis may not be appropriate. Indeed, both edentulous and periodontitis subjects have increased levels of serum CRP, which is a risk factor for CVD.[38] The relationship between edentulous status and increased risk of CVD is still unresolved.

We examined only the mesiobuccal and mid-buccal sites of all of the teeth in two randomly selected quadrants. However, Papapanou et al. reported that full mouth and partial estimates of the extent and severity of CAL were strongly correlated, and, in particular, the severity index (mean value of the sites with >1 mm CAL) was highly correlated ($r = 0.93$) in the 55- to 64-year-old age group.[39] We excluded subjects with <10 teeth from our study because of the inherent difficulties in properly assessing periodontal health in these patients. Takata et al. observed ECG abnormalities more frequently in octogenarians with fewer teeth.[40] Their study did not find a significant relationship between ECG abnormalities and periodontitis and they believed that this was due to the advanced age of their subjects, who had lost many teeth. Joshipura et al. reported that men with few teeth who reported preexisting periodontal disease had an increased risk of CVD.[41] Indeed, in our study, the number of missing teeth was one of the significant risk indicators relevant to ECG abnormalities in the bivariate analysis, although the significance disappeared after the multivariate evaluation, which suggests that periodontitis, rather than the loss of many teeth, was the true risk factor for CVD. The lack of consistency between studies of the relationship between periodontitis and CVD is attributed to differences in the