elsewhere (20, 21). Among a total of 418 subjects who attended the 2004 examination, 158 subjects (80 males and 78 females) who were 78 years old and had at least ten teeth with and without periodontitis were selected.

Periodontal examination

The periodontal examination included the assessment of probing depth (PD), attachment level (AL), and bleeding on probing (BOP). Parameters were measured at six sites on every tooth. Four trained dentists used calibrated pressured plastic periodontal probes set to give a probing force of 20 g and measured at 1-mm intervals. All functioning teeth were assessed except those that were partially erupted. A calibration of periodontal examination among all dentists was held, κ ranged from 0.81 to 1.00 for PD and from 0.74 to 1.00 for AL.

For the selection of subjects, two criteria for periodontal conditions were defined as follows. Model 1: periodontitis, having at least one tooth with a probing pocket depth

≥6mm; control, having teeth without a probing pocket depth ≥6mm. Model 2: periodontitis with bleeding, excluding subjects with ≤10% of sites with BOP from periodontitis in Model 1; control without bleeding, excluding subjects with >10% BOP from control.

A personal interview was conducted to obtain information regarding smoking habits. Body mass index (BMI) was calculated as an indicator of obesity, and subjects were divided into two groups by BMI: normal BMI (< 25.0) and high BMI (≥ 25). Fasting glucose levels were defined as either normal (< 110mg/dL) or high (≥ 110mg/dL).

ELISA assay

Blood samples were obtained in the morning for measurement of adiponectin, resistin, and other biochemical components. All sera were frozen and stored at -80°C until further measurement. Adiponectin, resistin, TNF-α, and IL-6 levels in serum samples were examined using an enzyme linked immunosorbent assay (ELISA) kit, KHP0041, KHP0051, KHC3014, and KHC0064 (Biosource International Inc., CA,

USA), respectively, according to the manufacture's protocol. In addition, each plate was checked before use to ensure that the calibration curve measuring the standard was accurate. All samples were run in duplicate. Absorbance of the substrate color reaction was measured using Microplate manager (Bio-Rad Laboratories, Hercules, CA, USA) at a primary wavelength of 405 nm.

Data analysis

Statistical analyses were conducted using SPSS version 12.0J (SPSS Japan, Tokyo, Japan). Quantitative data are presented as the mean ± standard deviation (SD) and the median. Statistical significance was estimated using either a chi-square test or an independent nonparametric test (Mann-Whitney U-test). Correlations were calculated using Spearman's rank correlations. Logistic regression analysis was performed to determine the association between periodontitis and the levels of serum resistin and adiponectin. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Each mean value was used as a cutoff point for high or low levels of

serum resistin and adiponectin. Adjusted mean value of serum resistin and adiponectin in the subjects with each periodontal condition were calculated by analyses of covariance (ANCOVA) adjusting for sex, smoking, BMI, and fasting glucose levels.

Results

Table 1 outlines characteristics of subjects in each periodontal conditions. In Model 1, there were significantly higher concentrations of total leukocytes (5.96 ± 1.43 vs. 5.46 ± 1.25×10^3/μL; p = 0.015) and neutrophils (57.78 ± 8.78 vs. 52.88 ± 9.96%; p = 0.001) in subjects with periodontitis compared to controls. There was a tendency of increased resistin levels and decreased adiponectin levels in periodontitis; however, this difference was not significant. Median values of TNF-α (0.71 vs. 0.60 pg/mL) and IL-6 (0.37 vs. 0.29 pg/mL) were higher in periodontitis patients than in control, albeit not significantly.

Model 2 showed a similar tendency, with significantly higher concentrations of

leukocytes (6.25 ± 1.64 vs. 5.44 ± 1.23×10^3/μL; p = 0.006) and neutrophils (59.09 ± 9.30 vs. 53.44 ± 9.97%; p = 0.004) in subjects with periodontitis with bleeding than in control without bleeding. Additionally, subjects with periodontitis showed significantly higher concentrations of resistin (6.10 ± 3.54 vs. 4.78 ± 2.95 ng/mL; p = 0.024) and higher BMI (not significant).

Furthermore, we conducted simple correlation analyses for all subjects between resistin and adiponectin and mean PD, mean AL, percentage of BOP, or leukocyte counts (Fig. 1). While resistin levels did not significantly correlate with mean PD and AL (Fig. 1A, 1B), they were positively correlated with BOP (r_s = 0.198, p = 0.013; Fig. 1C) and leukocyte counts (r_s = 0.233, p = 0.003; Fig. 1D). Adiponectin levels were negatively correlated with mean AL (r_s = -0.212, p = 0.007; Fig. 1F) and leukocyte counts (r_s = -0.316, p < 0.001; Fig. 1H), but not with mean PD (r_s = -0.154, p = 0.053; Fig. 1E) nor percentage of BOP (Fig. 1G). Serum levels of resistin and adiponectin were not significantly correlated with IL-6 and TNF-α (data not shown).

Logistic regression analysis was performed using higher resistin levels (≥

5.3ng/mL), and lower adiponectin levels (< 11.5ng/mL) as dependent variables. These cutoff points were determined using the mean serum levels of all subjects (Tables 2A and 2B). Sex, smoking, BMI, and fasting glucose levels were used as independent variables. Periodontitis was significantly associated with higher resistin levels both in Model 1 (OR, 2.0; 95%CI, 1.0-4.0) and in Model 2 (OR, 2.9; 95%CI, 1.2-6.9; Table 2A). BMI of ≥25 was associated with higher resistin levels only in Model 2 (OR, 3.2; 95%CI, 1.1-9.4). Although higher BMI negatively correlated with adiponectin as previously reported (r_s=-0.245, p=0.002), it was not significantly associated with decreased adiponectin levels in multivariate logistic regression analysis (Table 2B).

In an analysis of covariance for the same variables as above, i.e., sex, smoking, BMI, and fasting glucose levels, significantly higher resistin levels were observed in subjects with periodontitis with bleeding than in control without bleeding (4.78 ± 2.95 vs. 6.11± 3.54; Table 3). Adiponectin levels were slightly decreased in periodontitis and periodontitis with bleeding; however, these differences were not significant.

Discussion

Since all subjects used in this study were elderly (78 years old), individuals with at least one PD site ≥4mm made up the majority of subjects (86.1%). In addition, BOP levels (average: 10.9%, median: 7.0%) appeared to be much lower than in other reports (22). BOP is a reliable indicator of activity in periodontal disease (23) and may also indicate the progression of periodontal disease in community-dwelling elderly non-smokers (20). Therefore, lower BOP levels may indicate that many elderly people with deep PD and severe AL have a more stable periodontal condition compared to younger adults.

Among other inflammatory markers that are traditionally used as diagnostic measures to assess infection and inflammation, total leukocyte counts were significantly higher in subjects with periodontitis than in controls in this study. Moreover, our results revealed associations between resistin, adiponectin, and other inflammatory variables such as leukocyte counts and BOP. Specifically, resistin levels were siginificantly

correlated with BOP and leukocyte counts, indicating an existing inflammation. Therefore, we introduced BOP to the criteria of periodontitis and control in Model 2. In addition, serum resistin levels were weakly correlated with average PD, but not average AL. These results suggest that resistin levels may be associated with inflammatory variables rather than periodontal destruction such as indicated by AL. Although adiponectin levels were negatively correlated with mean AL, this result was most likely due to the lower adiponectin levels observed in males who had severe AL. Adiponectin levels were negatively correlated with leukocyte counts (Fig.1A), which indicates that adiponectin is an anti-inflammatory mediator as previously reported (24).

In contrast to the commonly held belief that serum IL-6 and TNF-α levels are increased in periodontitis, we found no significant relationship between either serum IL-6 or TNF-α levels and the severity of periodontitis. IL-6 and TNF-α locally delivered to the gingival tissues influence the pathogenesis of periodontal disease (1, 2). However, these cytokines may have little influence on circulating levels in elderly people. Additionally, TNF-α is produced mainly during the early stages of an acute inflammation,

and the production of TNF-α may be decreased in elderly people.

We found that resistin levels, but not adiponectin, were associated with periodontal condition and other inflammatory variables. Conversely, adiponectin levels were significantly higher in women (12.61 ± 4.95μg/mL) than in men (10.35 ± 6.04μg/mL, p =0.011), but resistin levels were not (women; 4.96 ± 2.71ng/mL, men; 5.53 ± 3.41ng/mL, p = 0.24). Therefore, we analyzed the relationship between serum adiponectin levels and periodontal condition in men and women separately. However, the results of these analyses did not reach statistical significance (data not shown). As in the case of previous study suggesting that adiponectin does not appear to be influenced by periodontal treatment (19), periodontal conditions were not associated with serum adiponectin levels in our study. Adiponectin levels might not be influenced by LPS stimulation in human, differently from leptin (25) and resistin (17, 25). Circulating adiponectin is present as several forms, including low, middle, and high molecular weight adiponectin, which may activate different signal transduction pathways and exert distinct effects (26). Some of these specific forms, such as high molecular weight adiponectin,

may be significantly associated with periodontal inflammation. Further studies examining the effects of high molecular weight adiponectin are necessary.

Studies have also indicated an abundance of resistin in peripheral blood mononuclear cells and macrophages, suggesting an important role of resistin in the process of inflammation (8, 10). Circulating resistin levels are elevated in patients with rheumatoid arthritis (27), cardiovascular disease (28, 29), and chronic kidney disease (30). In previous study, increased resistin promoted endothelial cell activation by endothelin-1 release and upregulates chemokines (28), suggesting that increased resistin related to cardiovascular disease. Increased resistin levels by periodontal inflammation may mediate the relationship between periodontitis and cardiovascular disease.

When BOP was introduced as part of the selection criteria of periodontal condition, the association between the two became stronger. Additionally, serum resistin levels were associated with periodontitis in middle-aged Japanese women (the Hisayama study, unpublished data). It remains unknown whether periodontal inflammation influences circulating resistin levels in humans; however, inflammatory

cells such as monocytes and macrophages present in the periodontal tissue appear to be the major source of resistin. Since inflammatory cytokines such as IL-6, TNF-α, and IL-1β have an effect on the expression of resistin *in vitro* (31), it is possible that periodontal inflammation influences resistin expression. Resistin expression also increases in concert with the maturation of monocyte into macrophages. Resistin may play a significant role in monocyte-macrophage function (10). In our study, adding monocyte as an independent variable in the logistic regression analysis did not affect the result. This result suggests that total monocytes may have a limited impact on resistin levels. However, when total leukocyte counts were added, the OR of periodontitis was reduced (data not shown). Total leukocyte counts including macrophage may be a causal intermediate existing between periodontitis and increased levels of resistin. Indeed, total leukocyte counts were significantly correlated both with mean PD (r_s=0.212, p=0.007) and percentage of BOP (r_s=0.205, p=0.01). Recent data indicate that stimulation of macrophages *in vitro* with LPS or proinflammatory cytokines leads to a marked increase in resistin production (17). Furthermore, administration of LPS to

humans is associated with dramatically increased circulating resistin levels (25). Therefore, it is possibile that LPS from periodontal pathogenic bacteria influences adipose tissues and macrophage through inflammatory cytokines.

In summary, here we report that serum resistin is associated with periodontal condition independent of sex, smoking, fasting glucose and BMI. Additionally, this association becomes stronger when BOP is included in the model. It is not clear how serum resistin is associated with periodontal inflammation. Further studies are required to clarify these mechanisms.

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Table 1. Characteristics of subjects with and without periodontitis

	Model 1[a]				
	Periodontitis (n=84)		Control (n=74)		P^b
	Mean ± SD	Median	Mean ± SD	Median	
Periodontal condition					
Mean of PD (mm)	2.52 ± 0.44	2.44	1.77 ± 0.21	1.76	<0.001
Mean of AL (mm)	3.45 ± 0.88	3.26	2.77 ± 0.63	2.78	<0.001
BOP (%)	15.60 ± 12.65	10.97	5.58 ± 7.32	3.25	<0.001
Adipokine&cytokine level					
Resistin (ng/mL)	5.58 ± 3.23	4.62	4.86 ± 2.90	4.01	0.131
Adiponectin(μg/mL)	10.92 ± 4.96	10.42	12.09 ± 6.27	10.66	0.199
TNF-α (pg/mL)	0.82 ± 0.74	0.71	1. 19 ± 2.20	0.60	0.954
IL-6 (pg/mL)	0.53 ± 0.61	0.37	0.86 ± 2.15	0.29	0.273
Blood components					
Leukocyte counts ($\times 10^3/\mu L$)	5.96 ± 1.43	5.70	5.46 ± 1.25	5.30	0.015
Platelet ($\times 10^4/\mu L$)	20.27 ± 4.15	19.55	20.14 ± 4.78	19.35	0.686
Monocyte (%)	6.21 ± 1.99	5.80	6.25 ± 1.72	6.10	0.650
Neutrophil (%)	57.78 ± 8.78	57.50	52.88 ± 9.96	52.10	0.001
General condition					
Male (%)	51.2		50.0		0.882
Smoking (%)[c]	46.4		43.2		0.690
BMI (kg/m^2)	22.77 ± 2.69	22.66	22.39 ± 2.56	22.20	0.384
Fasting glucose (mg/dL)	122.96 ± 39.73	114.0	118.37 ± 29.58	112.0	0.409
	Model 2[a]				
	Periodontitis with bleeding (n=47)		Control without bleeding (n=60)		P^b
	Mean ± SD	Median	Mean ± SD	Median	
Periodontal condition					
Mean of PD (mm)	2.65 ± 0.46	2.58	1.74 ± 0.20	1.75	<0.001
Mean of AL (mm)	3.64 ± 0.89	3.50	2.70 ± 0.60	2.75	<0.001
BOP (%)	23.62 ± 11.50	20.83	2.88 ± 2.67	1.93	<0.001
Adipokine&cytokine level					
Resistin (ng/mL)	6.10 ± 3.54	5.07	4.78 ± 2.95	3.94	0.024
Adiponectin(μg/mL)	10.85 ± 5.62	9.24	11.90 ± 6.55	10.66	0.283
TNF-α (pg/mL)	0.86 ± 0.80	0.84	1.22 ± 2.27	0.60	0.995
IL-6 (pg/mL)	0.59 ± 0.66	0.45	0.94 ± 2.31	0.32	0.272
Blood components					
Leukocyte counts ($\times 10^3/\mu L$)	6.25 ± 1.64	6.10	5.44 ± 1.23	5.30	0.006
Platelet ($\times 10^4/\mu L$)	20.92 ± 4.55	20.50	20.35 ± 4.91	19.45	0.444
Monocyte (%)	6.34 ± 2.36	5.80	6.29 ± 1.74	6.10	0.751
Neutrophil (%)	59.09 ± 9.30	57. 80	53.44 ± 9.97	52.35	0.004
General condition					
Male (%)	46.8		51.7		0.622
Smoking (%)[c]	42.6		43.3		0.936
BMI (kg/m^2)	23.01 ± 2.44	22.71	22.38 ± 2.46	22.20	0.145
Fasting glucose (mg/dL)	123.53 ± 43.00	113.0	117.23 ± 29.94	110.0	0.398

[a]In Model 1, selection criterion of periodontal condition was only with or without ≥6mm of probing depth, whereas in Model 2, 10% of BOP was considered in selection criterion in addition to probing pocket depth.
[b]*p*-values were calculated by Mann-Whitney U-test except for percentages of male and smoking by chi-square test.
[c] past or current smoking habit

Table 2A. Relationship between periodontal conditions and increased resistin level by logistic regression analysis

Model 1					
Independent variables	Resistin <5.3ng/ml	Resistin ≥5.3ng/ml	p^a	Multivariate OR[b] (95%CI)	p
Periodontal condition					
Control	54 (73.0)	20 (27.0)	0.066	1	
Periodontitis	49 (58.3)	35 (41.7)		2.00 (1.20-3.98)	0.046
Sex					
Male	50 (62.5)	30 (37.5)	0.507	1	
Female	53 (67.9)	25 (32.1)		0.43 (0.15-1.21)	0.109
BMI					
<25	87 (66.4)	44 (33.6)	0.510	1	
≥25	16 (59.3)	11 (40.7)		1.57 (0.64-3.85)	0.321
Fasting glucose					
<110mg/dL	43 (63.2)	25 (36.8)	0.736	1	
≥110mg/dL	60 (66.7)	55 (34.8)		0.77 (0.39-1.55)	0.466
Smoking habit					
No	56 (64.4)	31 (35.6)	0.867	1	
Yes	47 (66.2)	24 (33.8)		0.51 (0.12-1.42)	0.196
Model 2					
Independent variables	Resistin <5.3ng/ml	Resistin ≥5.3ng/ml	p^a	Multivariate OR[b] (95%CI)	p
Periodontal condition					
Control without bleeding	45 (75.0)	15 (25.0)	0.024	1	
Periodontitis with bleeding	25 (53.2)	22 (46.8)		2.90 (1.22-6.94)	0.016
Sex					
Male	34 (64.2)	19 (35.8)	0.840	1	
Female	36 (66.7)	18 (33.3)		0.35 (0.11-1.18)	0.091
BMI					
<25	60 (69.8)	26 (30.2)	0.074	1	
≥25	10 (47.6)	11 (52.4)		3.18 (1.08-9.38)	0.036
Fasting glucose					
<110mg/dL	30 (61.2)	19 (38.8)	0.422	1	
≥110mg/dL	40 (69.0)	18 (31.0)		0.57 (0.23-1.40)	0.219
Smoking habit					
No	38 (62.3)	23 (37.7)	0.539	1	
Yes	32 (69.6)	14 (30.4)		0.39 (0.12-1.28)	0.119

[a] chi-square test

[b] odds rario by logistic regression analysis