Serum Levels of Resistin and Adiponectin in Women with Periodontitis: the Hisayama Study

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ABSTRACT

Resistin and adiponectin, recently discovered adipokines, are secreted from adipose tissue, with postulated opposing functions in insulin resistance and inflammation. More recently, an abundance of resistin was detected in macrophages, which suggests its important role in inflammation. The aim of this study was to clarify circulating serum adipokine levels in women with periodontitis. Thirty-four women with moderate to severe periodontitis and 42 control individuals with healthy gingiva (50- to 59-year-old women) were selected. The serum level of adipokines was analyzed between groups, along with the obesity index, smoking status, and age. Having periodontitis was significantly associated with an increased level of resistin, both in bivariate (OR, 3.0; 95% CI, 1.2-7.6) and multivariate (adjusted OR, 3.1; 95% CI, 1.1-8.6) analyses. The association of periodontitis with a decreased level of adiponectin did not reach statistical significance. It was concluded that an increased serum resistin level in middle-aged Japanese women with periodontitis may affect systemic health.

KEY WORDS: periodontitis, resistin, adiponectin, adipokine, epidemiology.

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INTRODUCTION

Recent studies have suggested that obesity, type 2 diabetes, and periodontal disease are closely associated, exhibiting a triangular relationship (Saito and Shimazaki, 2007). Obesity is the greatest cause of type 2 diabetes (Uysal *et al.*, 1997; Kopelman, 2000), and periodontitis is the sixth most common complication of diabetes (Löe, 1993). Several studies have reported that periodontal treatment of persons with diabetes has a beneficial effect on glucose metabolism (Janket *et al.*, 2005), and that deep periodontal pocketing is associated with past glucose intolerance in non-diabetic community-dwellers (Saito *et al.*, 2004). Moreover, obesity and metabolic syndrome may be possible risk factors for periodontal disease (Saito *et al.*, 1998; Linden *et al.*, 2007; Saito and Shimazaki, 2007; Shimazaki *et al.*, 2007). By 75-g oral glucose tolerance tests, obesity has been found to be independently associated with periodontal disease in Japanese women, which suggests a direct effect of obesity on periodontal tissues (Saito *et al.*, 2005).

Before these complicated relationships can be clarified, studies on the causes are needed. The large quantity of adipose tissue that accumulates in obesity is becoming an important target for research on obesity-related disease. Adipose tissues secrete several bioactive molecules, such as leptin, adiponectin, and resistin, which are collectively called 'adipokines'. These products are secreted from adipose tissues and influence insulin resistance, and also play a role in inflammation and immune responses (Fantuzzi, 2005). Resistin and adiponectin are recently discovered adipokines with postulated opposing functions in insulin resistance (Pittas *et al.*, 2004). Resistin was first identified during a search for the antidiabetic drugs known as thiazolidinediones (TZDs) (Steppan *et al.*, 2001a,b). Serum levels of resistin are elevated in obese mice and lowered by treatment with TZDs. Therefore, resistin was initially described as an adipocyte-derived mediator of hepatic insulin resistance, and initial studies in rodents have suggested that resistin is up-regulated in obesity and might be involved in the development of insulin resistance (Steppan and Lazar, 2002). However, later studies have failed to confirm this hypothesis, and have shown reduced resistin expression in human adipose tissues (Lee *et al.*, 2003). Very low levels of resistin are found in human adipose tissues, but more is detected in peripheral blood mononuclear cells, macrophages (Patel *et al.*, 2003), and bone marrow (Shojima *et al.*, 2005), which suggests its possible role in inflammatory processes. Therefore, at present, resistin is thought to be linked to inflammatory, as well as metabolic, diseases (Fantuzzi, 2005).

In contrast, adiponectin decreases in obesity, type 2 diabetes, and cardiovascular diseases (Pittas *et al.*, 2004). Adiponectin has an anti-atherogenic function, improves insulin sensitivity, and reduces circulating fatty acids and triglycerides in muscle and liver tissue (Otero *et al.*, 2006). Moreover, adiponectin is also postulated to be associated with the modulation of inflammatory responses. It attenuates the inflammatory response mediated by tumor necrosis factor α (TNF-α), and inhibits

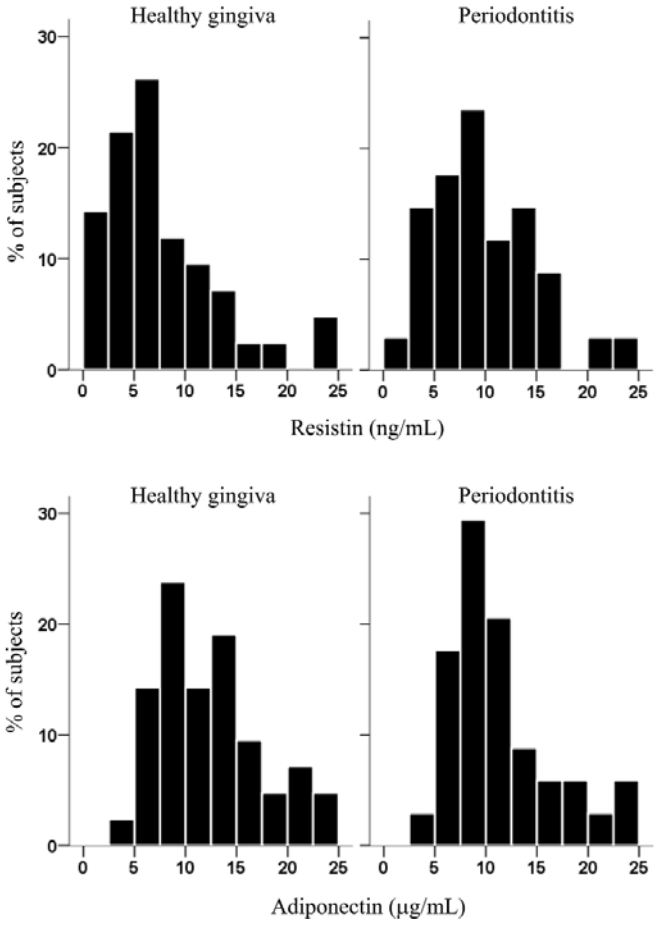

Figure. Percentage distribution of serum levels of adipokines in women with and without periodontitis. N = 42 for healthy gingiva and N = 34 for periodontitis. Distributions of resistin and of adiponectin between groups were not significantly different by chi-square test. Mean and standard deviation of each group are represented in Table 1.

macrophage phagocytic activity and TNF-α production (Yokota *et al.*, 2000). We have recently reported that adiponectin negatively regulates macrophage-like cell responses to Toll-like receptor ligands (Yamaguchi *et al.*, 2005), and inhibits osteoclast formation stimulated by lipopolysaccharide from *Aggregatibacter actinomycetemcomitans* (Yamaguchi *et al.*, 2007).

Based on these recent studies, we postulated that resistin and adiponectin may be candidates for explaining the triangular relationship among obesity, type 2 diabetes, and periodontal disease. The purpose of this study was to clarify the relationship between these adipokines and periodontal disease by comparing women with periodontitis and those with healthy gingiva.

MATERIALS & METHODS

The Hisayama study began in 1961 and is an ongoing population-based prospective cohort study of cardiovascular diseases. As a part of the study, from July to August, 2004, a comprehensive health examination, including a periodontal examination, was conducted. The periodontal examination was planned for the residents 40, 50 to 59, 60, 70, and 80 yrs old. Those residents received questionnaire sheets by mail from the Hisayama town office, and provided written informed consent. Finally, 743 participants, including other residents who wanted dental examinations, underwent periodontal examinations. Among them, 402 participants were from 50 to 59 years old (161 men and 241 women). Serum levels of adipokines differ frequently between sexes and sometimes between age groups. Considering the masking effect of smoking, the rate of which was considerably higher in men than in women, we selected women 50 to 59 yrs of age as a homogeneous group of participants in this case-control pilot study. In accordance with the method of the Third National Health and Nutrition Examination Survey (NHANES III) (Brown *et al.*, 1996), a periodontal examination was performed on all existing teeth by four trained dentists using a standard dental chair. Examiners were calibrated in advance. Periodontitis was defined as the presence of at least 1 tooth with ≥ 6 mm of probing pocket depth and/or at least 3 teeth with ≥ 4 mm of probing pocket depth. Healthy gingiva was defined as the presence of all teeth with ≤ 3 mm probing pocket depth and no more than 2 teeth that bled when probed. Of the 241 women examined, 76 apparently healthy women with at least 22 teeth each were finally selected; 34 exhibited moderate-to-severe periodontitis (case individuals), and 42 exhibited healthy gingiva (control individuals).

Blood samples were collected from the antecubital vein on the morning after an overnight fast, and sera were stored in a deep freezer, as previously described (Kubo *et al.*, 1999). Resistin, adiponectin, TNF-α, and IL-6 in peripheral blood were examined with the use of an enzyme-linked immunosorbent assay (ELISA) kit (RD191016100; BioVendor Laboratory Medicine, Brno, Czech Republic), a Human Adiponectin ELISA Kit (Otsuka Pharmaceutical Co., Tokyo, Japan), IM11121 (Beckman Coulter, Paris, France), and IM11120 (Beckman Coulter), respectively.

Body mass index (BMI, weight divided by the square of the height), waist circumference, waist-hip ratio, and body fat (bio-impedance method; TANITA Co., Tokyo, Japan) were measured. Smoking status was determined by the number of cigarettes smoked *per* day, multiplied by the number of years smoked. Only seven women were current or past smokers. The smoker rate was a little lower than the national average of the same age group (16.4%).

Statistical analysis was performed by simple comparison between women with periodontitis and those with healthy gingiva, by the Mann-Whitney *U* test. We used logistic regression analysis to determine the effect of periodontitis on the levels of serum adipokines and IL-6, and calculated the odds ratio (OR) and 95% confidence interval (CI). Each median value was used as a cut-off point for high or low levels of serum adipokines and IL-6, with consideration for possible skewed distributions of these substances. SPSS version 15.0 (SPSS Japan, Tokyo, Japan) was used for the analysis. Written informed consent was obtained from all participants before examination. 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, Japan.

RESULTS

The distributions of serum levels of resistin and adiponectin are represented in comparison between women with healthy gingiva and those with periodontitis (Fig.). Adipokines and other factors were compared between the women with healthy gingiva and those with periodontitis (Table 1). Among these adipokines, resistin alone was significantly higher in the women with periodontitis (P = 0.037). Serum levels of

adiponectin in the women with periodontitis were slightly lower than in those with healthy gingiva, but the difference was not significant. TNF-α and IL-6 showed no significant difference between the groups (data not shown).

BMI was significantly higher in the women with periodontitis (P = 0.013). Other variables of obesity were higher in the women with periodontitis, but the differences were not significant.

We divided the participants into two groups, depending on high or low serum resistin level, using the median level of serum resistin in all participants (7.5 ng/mL) as a cut-off point. Logistic regression analysis was performed with periodontal disease (yes/no), BMI (≥ 25 *vs.* < 25), and age (continuous) as independent variables, and resistin level as a dependent variable (Table 2). Having periodontitis was significantly associated with an increased level of resistin in both the bivariate (OR, 3.0; 95% CI, 1.2-7.6) and multivariate (adjusted OR, 3.1; 95% CI, 1.1-8.6) analyses. Higher age was significantly associated with lower resistin level. BMI ≥ 25 was not significantly associated with the resistin level.

The relationship between periodontitis and serum adiponectin level was analyzed in the same manner. Having periodontitis was associated with a decreased level of adiponectin, but the results were not significant (adjusted OR, 1.7; 95% CI, 0.7-4.4; P = 0.28). Women with a high BMI also showed a decreased level of adiponectin, but the results also did not reach statistical significance (adjusted OR, 2.4; 95% CI, 0.7-8.1; P = 0.15). Age was not associated with the serum adiponectin level.

Table 1. Comparison between Women with and Those without Periodontitis

	Participants with Healthy Gingiva, N = 42		Participants with Periodontitis, N = 34		
	mean ± SD	median	mean ± SD	median	P[a]
Adipokines					
Resistin (ng/mL)	8.0 ± 5.2	6.7	9.9 ± 5.0	9.1	0.037
Adiponectin (μg/mL)	12.4 ± 5.1	11.8	11.5 ± 5.0	10.0	0.46
Other variables					
BMI[b] (kg/m^2)	21.9 ± 3.2	21.8	23.3 ± 2.6	23.4	0.013
Body fat (%)	26.4 ± 6.0	26.5	28.7 ± 4.6	29.6	0.079
Waist (cm)	78.9 ± 10.7	79.5	81.0 ± 8.0	81.3	0.39
Waist-hip ratio	0.89 ± 0.06	0.90	0.89 ± 0.07	0.89	0.92
Smoking	23.4 ± 149.9	0	40.7 ± 131.2	0	0.28
Age (yrs)	55.1 ± 2.7	55	55.2 ± 2.4	55	0.85

[a] Mann-Whitney *U* test.
[b] Body mass index: weight divided by the square of the height.

Table 2. Relationship between Periodontal Disease and Increased Serum Level of Resistin in Japanese Women

	Resistin (ng/mL)		Bivariate Analysis		Multivariate Analysis	
Variable	< 7.5	≥ 7.5	OR (95%CI)	P	Adjusted OR (95%CI)	P
Periodontal disease	Number of Participants (%)					
No	26 (61.9)	16 (38.1)	1		1	
Yes	12 (35.3)	22 (64.7)	3.0 (1.2-7.6)	0.023	3.1 (1.1-8.6)	0.026
BMI[a]						
< 25	32 (53.3)	28 (46.7)	1		1	
≥ 25	6 (37.5)	10 (62.5)	1.9 (0.6-5.9)	0.27	1.7 (0.5-5.8)	0.38
Age (continuous)	55.8 ± 2.4[b]	54.5 ± 2.6[b]	0.8 (0.7-1.0)	0.028	0.8 (0.6-1.0)	0.018

[a] Body mass index: weight divided by the square of the height.
[b] Mean ± standard deviation.

DISCUSSION

Adipokines are believed to act through their effects on insulin sensitivity; moreover, new lines of evidence indicate an important effect on the inflammatory process (Fantuzzi, 2005). The activation of inflammatory mechanisms was detected in adipose tissue, and it was proposed that these mechanisms are involved in the development of complications of obesity. The role of resistin in insulin resistance and diabetes has been well-established in mice (Steppan and Lazar, 2002), but in rats and humans, its role and relationship are controversial. However, recent studies indicating an abundance of resistin in peripheral blood mononuclear cells and macrophages suggest an important role for resistin in the inflammatory process (Patel *et al.*, 2003). The circulating resistin level is elevated in persons with rheumatoid arthritis (Migita *et al.*, 2006), cardiovascular disease (Takeishi *et al.*, 2007), chronic kidney disease (Yaturu *et al.*, 2007), non-alcoholic fatty liver (Pagano *et al.*, 2006), and diabetic retinopathy (Osawa *et al.*, 2007). Moreover, periodontitis is a common chronic subclinical inflammation of the periodontal tissues, with widespread distribution of Gram-negative bacteria in deep pockets. The increase in circulating resistin levels shown in our study may result from the local involvement of monocytes and macrophages in periodontal inflammation.

Stimulation of macrophages *in vitro* with endotoxin or pro-inflammatory cytokines leads to a marked increase in resistin production. Furthermore, administration of endotoxin to humans is associated with dramatically increased circulating resistin levels (Lehrke *et al.*, 2004). Lipopolysaccharide from periodontal Gram-negative pathogens such as *Porphyromonas gingivalis* might stimulate adipose tissues, which are composed of adipocytes and the stromavascular fraction including macrophages. Thus, the increase in circulating resistin levels

induced by periodontal disease might be derived from both periodontal and adipose tissues.

In this study, the difference between the serum level of adiponectin in both the case and control participants was small, and the relationship between periodontitis and the lower 50th percentile of adiponectin did not reach statistical significance. The correlation between adiponectin, but not resistin, and BMI was significant. Therefore, the weak relationship between adiponectin and periodontitis might have arisen due to a relationship between BMI and periodontal disease. Recent studies showed that the high-molecular-weight (HMW) form of adiponectin is the most active form of this hormone. The HMW ratio (ratio of the plasma level of HMW adiponectin to that of total adiponectin) compared with the total adiponectin level is better able to predict insulin resistance and metabolic syndrome (Hara *et al.*, 2006). Analysis with the HMW form of adiponectin may yield significant results. Although we also examined the TNF-α and IL-6 levels, we did not find a significant relationship between these and periodontitis. Since we did not use high-sensitivity ELISA kits for TNF-α and IL-6, in many women, these levels were below the lower detection limits of the kits. Since we selected homogeneous characteristics for both case and control participants, the numbers of our participants were limited, especially those with severe periodontitis. Further studies with larger sample size are necessary.

In conclusion, the serum resistin level was associated with periodontitis in middle-aged Japanese women. Resistin may mediate the triangular relationship that exists among obesity, type 2 diabetes, and periodontal disease.

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