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Original article

Association of low fetuin-A levels with periodontitis in community-dwelling adults

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Abstract: Fetuin-A is a liver-secreted glycoprotein isolated from fetal bovine serum. Recent reports of its several pathological functions suggest an association between fetuin-A and systemic diseases. This study therefore examined the correlation between serum fetuin-A level and periodontal status. Data from 356 middle-aged and elderly adults who underwent health examinations in Goto, Japan, during the period from 2008 through 2010 were analyzed. Systemic and periodontal measurements were recorded, and serum fetuin-A level was determined by using an enzyme-linked immunosorbent assay. Fetuin-A levels for participants with moderate to severe periodontitis were significantly lower than those for participants with no or mild periodontitis. Additionally, fetuin-A level negatively correlated with periodontal clinical attachment loss. Moderate to severe periodontitis was significantly correlated with low serum fetuin-A levels (odds ratio, 1.69; 95% confidence interval, 1.01-2.69) in logistic regression analysis. Low serum fetuin-A level was correlated with worse periodontal status and could thus potentially serve as a marker of periodontitis.

Keywords; epidemiology, fetuin-A, periodontitis

Introduction

Periodontitis, chronic inflammation of the supporting tissues around the teeth, is a common disease. Inflamed periodontal pockets facilitate entry of oral microorganisms [1]. The host immune response against periodontal pathogens, including cytokines associated with inflammation, is believed to have an important role in periodontitis [2]. Risk factors for periodontitis include host systemic diseases such as dyslipidemia, atherosclerosis, and type 2 diabetes, which is associated with obesity [3-5].

Fetuin-A is a liver-produced glycoprotein present at high concentrations in blood [6]. It has important effects on regression of bone metabolism, in metabolic disorders such as type 2 diabetes, and in calcification in atherosclerosis [7] and is significantly lower in persons with coronary artery calcification [8], coronary heart disease, and rheumatoid arthritis [9]. A previous study reported that periodontitis was associated with lower circulating levels of fetuin-A [10].

Previous studies found that adipokines linked to obesity, such as resistin and adiponectin, were associated with periodontitis [11,12]. Biomarker substances such as adipokines and cytokines can be measured objectively and are indicators of pathogenic processes. Considerable evidence indicates that serum biomarkers reflect the extent of periodontal tissue destruction and predict disease progression in periodontitis [13].

The present study examined the possible link of fetuin-A level with periodontal status and systemic blood parameters in a community-based population and evaluated potential associations between fetuin-A, systemic blood markers, and risk of periodontal progression.

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Materials and Methods

Participants

The present study was approved by the Ethics Committee of Nagasaki University (project number 090528160) before the study began. This cross-sectional study was conducted in accordance with the Declaration of Helsinki and analyzed data from 356 adults (105 men and 251 women), aged 40 years or older, who participated in the "Special Health Check-up" and oral assessment in Goto City, Japan. Participants were excluded if they had fewer than eight teeth. All participants provided written informed consent to take part in this study and answered a detailed medical questionnaire. Smoking habit was categorized as current/past and never smoker.

Periodontal disease examination

Periodontal status was determined by measuring pocket depth (PD) and clinical attachment loss (CAL) with a periodontal probe at two sites (mesiobuccal and midbuccal) per tooth, except for wisdom teeth [11]. Bleeding on probing (BOP) was determined at 15 to 30 s after probing. Plaque score was recorded by using the simplified debris index (DI-S) [14]. Four experienced dentists used periodontal probes and measured PD with a light force of 20 gf to obtain measurements in millimeters. Periodontal status was classified as healthy to mild and moderate to severe, in accordance with the classification detailed by the Centers for Disease Control and Prevention jointly with the American Academy of Periodontology [15].

Data collection

Each participant underwent measurement of body weight and height to calculate body mass index (BMI; kg/m^2). Systolic and diastolic blood pressures (SBP and DBP) were measured under resting conditions. An SBP of ≥130 mm Hg and/or a DBP of ≥85 mm Hg was regarded as hypertension. Blood samples were collected from each participant after an overnight fast. Separated serum from blood was preserved at −20°C until analysis. Triglyceride (TG) level was quantified with enzymatic methods, high-density lipoprotein cholesterol (HDL-C) was quantified with direct methods, low-density lipoprotein cholesterol (LDL-C) was estimated by using the Friedewald equation, and hemoglobin A1c (HbA1c) level was quantified by latex agglutination.

Enzyme-linked immunosorbent assay (ELISA)

Fetuin-A levels were determined by using an ELISA kit (R&D Systems, Minneapolis, MN, USA) in accordance with the established protocol. Each sample and standard dilutions were measured in duplicate. Absorbance of the substrate color development was analyzed with Microplate Manager (Bio-Rad Laboratories, Hercules, CA, USA).

Data analysis

Statistical analyses were performed with the software package (IBM SPSS Statistics ver.21, IBM Japan, Tokyo, Japan). Continuous and categorical variables are expressed as mean ± SD and as number (%), respectively. Statistical comparison of groups was done with the nonparametric Mann-Whitney *U* test and chi-square test. Spearman rank correlation coefficients were used to assess correlations between fetuin-A, periodontal variables, and systemic variables. Relationships of periodontitis with multiple variables were analyzed with logistic regression analyses, after adjustment for covariates. Odds ratios (ORs) and 95% confidence intervals are presented. A P value of <0.05 was considered to indicate statistical significance.

Table 1 Clinical characteristics of participants, according to periodontal status

	Healthy/mild (n = 140)	Moderate/severe (n = 216)	P value
Fetuin-A (μg/mL)	158.7 ± 104.3	128.7 ± 100.1	0.005 [a]
Sex (Female / Male)	116/24 (82.9/17.1)	135/81 (62.5/37.5)	< 0.001 [b]
Age (years)	60.9 ± 11.3	65.9 ± 9.2	< 0.001 [a]
BMI (kg/m^2)	22.6 ± 3.2	23.0 ± 3.2	0.171 [a]
SBP (mmHg)	131.7 ± 19.5	138.1 ± 19.3	0.002 [a]
DBP (mmHg)	78.6 ± 9.7	81.1 ± 10.7	0.044 [a]
TG (mg/dL)	98.5 ± 52.0	104.3 ± 75.8	0.990 [a]
HDL-C (mg/dL)	63.5 ± 14.8	60.8 ± 15.4	0.057 [a]
LDL-C (mg/dL)	120.2 ± 32.0	120.0 ± 29.1	0.852 [a]
HbA1c (%)	5.3 ± 0.5	5.3 ± 0.5	0.694 [a]
Mean PD (mm)	1.4 ± 0.4	1.7 ± 0.6	< 0.001 [a]
Mean CAL (mm)	2.0 ± 0.4	3.1 ± 0.9	< 0.001 [a]
BOP (%)	12.7 ± 16.0	20.2 ± 19.1	< 0.001 [a]
DI-S	0.7 ± 0.6	1.0 ± 0.7	< 0.001 [a]
Smoking habit (%)	19/140 (13.6)	54/216 (24.9)	0.009 [b]

Values are means ± SD for continuous data. Values are numbers (%) for categorical data. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; PD, pocket depth; CAL, clinical attachment loss; BOP, bleeding on probing; DI-S, simplified debris index; Smoking habit, current or past smoker /never smoker. [a]Mann-Whitney U test. [b]Chi-square test

Table 2 Correlations of fetuin-A with clinical periodontal and systemic variables

Variables	ρ	P value
Age	−0.093	0.08
BMI	−0.041	0.45
SBP	−0.025	0.64
DBP	−0.035	0.51
TG	0.061	0.25
HDL-C	0.041	0.44
LDL-C	0.067	0.21
HbA1c	0.149	0.06
Mean PD	−0.069	0.20
Mean CAL	−0.131	0.01
BOP	0.018	0.73
DI-S	−0.250	0.64

ρ, Spearman rank correlation coefficient. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; PD, pocket depth; CAL, clinical attachment loss; BOP, bleeding on probing

Results

Among the 356 study participants, mean age was 60.9 ± 11.3 years in the control group and 65.9 ± 9.2 in persons with moderate to severe periodontitis (Table 1). The proportion of past/current smokers was significantly higher among participants with moderate to severe periodontitis than among the controls (P <0.001). Serum fetuin-A levels were significantly lower in those with moderate to severe periodontitis than in those with no or mild periodontitis. SBP and DBP were significantly higher in persons with moderate to severe periodontitis. HDL-C was lower in those with moderate to severe periodontitis, but the difference was not significant. Analysis of clinical oral health variables showed that PD, CAL, BOP, and DI-S values were significantly higher in persons with moderate to severe periodontitis than in the controls.

Table 2 shows the correlations of serum fetuin-A level with other variables. Fetuin-A was significantly negatively correlated with CAL (ρ = −0.131, P = 0.01) and nonsignificantly positively correlated with HbA1c (ρ = 0.149, P = 0.06).

After adjustment for age, sex, BMI, HDL-C, smoking habit, hypertension, and DI-S, the variables significantly associated with periodontitis were male sex (OR 2.03, P = 0.013), low serum fetuin-A level (OR 1.69, P = 0.038), and a DI-S score of ≥1 (OR 2.69, P = 0.007) (Table 3). Age, smoking habit, and hypertension status were significantly associated with periodontitis in the crude analysis but not after adjustment for periodontitis risk factors.

Discussion

Age, SBP, DBP, and past/current smoking status were linked to periodontitis in previous studies [16,17] and significantly differed between the no/mild and moderate/severe periodontitis groups in the present study (Table 1). In addition, serum fetuin-A levels were significantly lower for moderate/severe periodontitis than for no/mild periodontitis (Table 1), which was also the case in a previous study [10]. A previous study reported that fetuin-A levels in serum and gingival crevicular fluid significantly decreased in relation to periodontitis progression [10].

In this study, fetuin-A levels were nonsignificantly negatively correlated with age. The analysis included middle-aged and elderly community-dwelling adults who underwent a general health examination. Although a previous study reported that fetuin-A was positively correlated with BMI, SBP, DBP, and HDL-C in middle-aged populations [18], this was not observed in the present study (Table 2). The present population-based study was performed in Goto, Japan, where aging and depopulation are accelerating.

Fetuin-A was significantly negatively correlated with mean CAL (Table 2). By regulating transforming growth factor and bone morphogenic protein, fetuin-A has potent osteogenic and differentiation effects [19]. A previous study reported that fetuin-A inhibited calcification in cultured osteoblasts [20]; however, a recent study found that fetuin-A treatment had a protective effect on bone resorption in a murine calvaria model of osteolysis [21]. Thus, studies of the effects of fetuin-A on bone mineralization have yielded contradictory findings. The present results suggest that low serum fetuin-A levels facilitate alveolar bone resorption.

BOP is an early predictor of gingivitis and is associated with periodontal inflammation. A previous study reported that the association of periodontitis with serum resistin levels was stronger when BOP was present [12]. In this study, BOP was not correlated with fetuin-A. BOP may not be an additional effect on the association of periodontitis to fetuin-A.

Fetuin-A is abundant, but its roles in disease progression are poorly understood. Previous studies reported that fetuin-A is crucial in lipid metabolism [22]. In this study, fetuin-A was not correlated with HDL-C or LDL-C, although HDL-C was nonsignificantly lower in persons with moderate/severe periodontitis. Fetuin-A is down-regulated by several pro-inflammatory molecules, including tumor necrosis factor-α, interleukin-6, andinterferon-γ [23]. These cytokines are induced by periodontal inflammation and affect periodontitis pathogenesis. Moreover, these cytokines might be associated with fetuin-A, and this association may be linked to periodontal progression.

In logistic regression analysis, advanced periodontitis was significantly associated with a low fetuin-A level (OR 1.66) and male sex (OR 2.03). Salivary fetuin-A levels were higher after periodontal treatment than at baseline in a previous study [24]. Future studies should thus examine whether serum or salivary fetuin-A level is a potential biomarker of peri-

Table 3 Association of periodontal status and low fetuin-A level in logistic regression analysis

Independent variables	Healthy/ Mild	Moderate/ Severe	*P* value	Multivariate OR (95% Cl)	*P* value
Age (years)					
<65	84 (47.2%)	94 (52.8%)	0.002	1	
≥65	56 (31.5%)	122 (68.5%)		1.48 (0.93-2.38)	0.101
Sex					
Female	116 (46.2%)	135 (53.8%)	< 0.001	1	
Male	24 (22.9%)	81 (77.1%)		2.03 (1.17-3.55)	0.013
Fetuin-A (μg/mL)					
≥73 (3rd tertile)	36 (30.3%)	83 (69.7%)	0.013	1	
<73 (-2nd tertile)	104 (43.9%)	133 (56.1%)		1.69 (1.03-2.76)	0.038
BMI					
<25 (kg/m^2)	109 (40.5%)	161 (59.5%)	0.417	1	
≥25 (kg/m^2)	31 (35.6%)	56 (64.4%)		0.92 (0.53-1.60)	0.766
HDL-C					
>40 (mg/dL)	137 (39.9%)	206 (60.1%)	0.222	1	
≤40 (mg/dL)	3 (23.1%)	10 (76.9%)		0.65 (0.16-2.68)	0.553
Smoking habit					
Never	121(42.8%)	163 (57.2%)	0.009	1	
Current/past	19 (26.0%)	54 (74.0%)		1.19 (0.56-2.52)	0.659
Hypertension					
Yes	67 (45.6%)	80 (54.4%)	0.043	1	
No	73 (34.9%)	136 (65.1%)		1.34 (0.83-2.17)	0.231
DI-S					
<0.33 (1st quartile)	35 (49.3%)	36 (50.7%)	< 0.001	1	
0.5-0.8 (2nd quartile)	57 (48.7%)	60 (51.3%)		1.04 (0.57-1.90)	0.906
0.8-1.0 (3rd quartile)	29 (36.7%)	50 (63.3%)		1.45 (0.73-2.85)	0.289
1.0≥ (4th quartile)	19 (21.3%)	70 (78.7%)		2.69 (1.31-5.53)	0.007

P value calculated by chi-square test or logistic regression analysis. OR, odds ratio; CI, confidence interval; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; Smoking habit, never smoker/ current or past smoker; DI-S, simplified debris index

odontitis.

This study has some limitations. First, because it was done in a community with accelerating aging and depopulation, it might be inappropriate to generalize the present findings to other populations. Second, the cross-sectional study design does not permit inference of causal or direct associations. Therefore, larger, long-term cohort studies will be necessary in order to examine the cause-effect relationship between fetuin-A and periodontitis.

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Conflict of interest

The authors have no conflict of interest to declare.

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