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Number of teeth and serum lipid peroxide in 85-year-olds

K. Sonoki[1], Y. Takata[1], T. Ansai[2], K. Fujisawa[1], M. Fukuhara[1], M. Wakisaka[1], S. Akifusa[2], I. Soh[2], A. Yoshida[2], T. Hamasaki[2], S. Awano[2], and T. Takehara[2]

Division of [1]General Internal Medicine and [2]Community Oral Health Science, Department of Health Promotion, Science of Health Improvement, Kyushu Dental College, Kitakyushu, Japan

Objectives To investigate influence of dental status on systemic oxidative stress, we evaluated the association between number of teeth and serum lipid peroxide, an oxidative stress index, in 85-years old residents of Japan. ***Methods*** In October 2003, 207 subjects 85-years old agreed to participate in the present follow-up study after five years from the 8020 Data Bank Survey of Fukuoka prefecture in 1998. Dental health condition including number of teeth was examined by dentists. Data from 204 subjects (88 male, 116 female) who completed nonfasting venous blood examination including lipid peroxide and blood chemistry were analyzed. The examination included a medical questionnaire regarding smoking history, physical activity, alcohol consumption, educational duration, and regular dental care, anthropometric and manometric measurements. ***Results*** Albumin, lipids, and lipid peroxide in serum all were within the normal range. Number of teeth correlated positively with height and white blood cell count, and correlated negatively with lipid peroxide. In a multiple regression analysis to adjust for confounding factors, tooth number retained this correlation with lipid peroxide. By analysis of variance with a Bonferroni-Dunn correction, edentulous subjects showed significantly higher lipid peroxide than those retaining 20 teeth or more. ***Conclusion*** The negative association between number of teeth and lipid peroxide links more teeth remaining with less oxidative stress in an 85-year-old population; this may decrease risk of atherosclerotic complications.

Key words: Lipid peroxide, number of teeth, oxidative stress, 85-year-olds

Introduction

Dental status including local infection (Mattila *et al*., 1995), caries (Yoshihara *et al*., 2003), and tooth loss (Marshall *et al*., 2002) is increasingly recognized to influence general health. Tooth loss, which is common during aging, compromises mastication of food by elderly people and ineffective chewing may negatively influence nutritional status (Walls *et al*., 2000). We also have reported a positive relationship between number of teeth and physical activity (Ansai *et al*., 2000), a relationship between tooth loss and electrocardiographic abnormalities (Takata *et al*., 2001), an association of number of teeth with heart rate (Matsumura *et al*., 2003), and a relationship of physical fitness to chewing ability (Takata *et al*., 2004). All were found in an 80-year-old Japanese population participating in a cross-sectional survey (8020 Data Bank Survey) in 1998.

In 2003, after five years, we set out to compare data concerning systemic and dental health conditions in 85-year-old subjects with the baseline data obtained when they were 80-years old. In addition, we were interested in the relationship between dental status and oxidative stress, which presently is considered a major contributor to atherosclerosis including cardiovascular disease (Halliwell, 1994). Lipid peroxide is a well-known oxidative stress marker that can be measured by readily available methods such as testing for thiobarbituric acid-reactive substances (TBARS) (Yagi, 1987). Elevated concentrations of lipid peroxide have been found in aging and diabetic patients, particularly those with angiopathy (Yagi, 1987), hyperlipidemia (Chirico *et al*., 1993), and ischemic heart disease (Stringer *et al*., 1989).

On the other hand, important aspects of dental status such as periodontal disease (Iacopino and Culter, 2000) and number of teeth (Norlen *et al*., 1996) recently have been found to be related to serum lipid homeostasis. The elevation of serum lipids, that is, hyperlipidemia leads to lipid peroxidation increasing oxidative stress (Chirico *et al*., 1993), and in fact, patients with periodontitis have been reported to have significantly higher concentrations of TBARS than healthy subjects (Panjamurthy *et al*., 2005). However, up to now, whether the number of remaining teeth is related to lipid peroxidation has been uncertain. Accordingly, to examine links between dental status and oxidative stress in 85-year-old subjects, we investigated the association between number of teeth and serum lipid peroxide concentration.

Materials and Methods

Of 827 participants in the 8020 Data Bank Survey in 1998, 207 subjects -- now 85 years old -- agreed to participate in the present survey in October 2003. The 8020 Data Bank Survey in Fukuoka prefecture of Japan was a community-based cross-sectional survey of 80-year-old residents as previously reported (Ansai *et al*., 2000, Takata *et al*., 2001 and 2004, Matsumura *et al*., 2003). In the present follow-up study, data were obtained from 204 subjects (88 male, 116 female) from whom a blood

Correspondence to: Dr. Kazuo Sonoki, Division of General Internal Medicine, Department of Health Promotion, Science of Health Improvement, Kyushu Dental College, Manazuru 2-6-1, Kokurakita-ku, Kitakyushu, 803-8580, Japan. E-mail: sonoki@kyu-dent.ac.jp

sample was taken for analysis. The study was approved with respect to the rights and treatment of subjects by the Human Ethics Committee of Kyushu Dental College, and informed consent was obtained from all participants.

The examination included completion of a medical questionnaire including questions regarding smoking history, physical activity, alcohol consumption, years of education completed, regular dental care, and number of chewable foods. Dental health conditions including number of teeth were examined by dentists. These examiners performed all oral examinations using criteria recommended by the World Health Organization (WHO, 1997). In determining the number of remaining teeth, root tips were counted among teeth present. All participants including those who were edentulous or had numerous missing teeth were examined regarding prosthetic condition to determine whether they had a bridge, partial denture, or full denture. As for denture stability and retention, occlusion status with and without the denture was evaluated using the Eichner Index. The number of chewable foods was determined by a questionaire, in which each subject was asked about ability to chew the following 15 foods; peanuts, yellow picked radish, hard rice cracker, French bread, beefsteak, vinegared octopus, pickled shallots, dried scallops, dried cuttlefish, squid sashimi, konnyaku, a tubular roll of boiled fish paste, boiled rice, tuna sashimi, and grilled eel, as we previously reported (Takata *et al*., 2004).

Nonfasting venous blood samples were obtained. Physical examination was performed including measurement of height, weight, and blood pressure. Patients with diabetes mellitus were defined as those being treated by local physicians with diet and/or hypoglycemic agents; diabetes was confirmed by blood determinations showing more than 200 mg/dl of glucose or more than 6.5% of hemoglobin (Hb) A1c. Patients with hypertension were defined as those being treated by local physicians with antihypertensive medication or showing more than 160 mm Hg for systolic blood pressure or more than 90 mm Hg for diastolic blood pressure. Hyperlipidemia was defined as treatment by local physicians with antihyperlipidemic agents, a total cholesterol concentration more than 220 mg/dl, or a triglyceride concentration more than 150 mg/dl. In addition, gender, smoking (current smoker vs. nonsmoker), alcohol consumption (yes vs. no), regular outpatient dental care (yes vs. no), regular exercise (yes vs. no), and duration of education (years) were recorded. Alcohol consumption was defined to exclude very infrequent, slight consumption. Regular outpatient dental care was defined as more than one dental visit per year. Regular exercise was defined by an affirmative answer to the question, “Do you presently exercise?”.

All clinical laboratory values including blood cell counts and blood biochemical examination were measured by a commercial laboratory (SRL, Tachikawa; Tokyo, Japan). TBARS was measured as lipid peroxide according to Yagi (1987), with the normal concentration being 1.8 to 4.7 nmol/ml, by a commercial laboratory (SRL) .

Differences between two groups were examined with the Mann-Whitney U test. Spearman’s rank correlation was used to evaluate the relationship between number of teeth and clinical laboratory parameters. Multiple regression analysis was carried out to evaluate whether number of teeth was related to lipid peroxide after adjustment for confounding variables such as gender, body mass index (BMI, kg/m^2), systolic blood pressure (mm Hg), total cholesterol (mg/dl), hemoglobin (Hb) A1c (%), white blood cell count (/μl), smoking, alcohol consumption, regular exercise, and regular outpatient dental care. Subjects were divided into four groups according to number of remaining teeth: group 1, with 20 or more (n = 27); group 2, with 10 to 19 (n = 48); group 3, with 1 to 9 (n = 50); and group 4, edentulous (n = 79), as in our previous reports (Takata *et al*., 2004; Matsumura *et al*., 2003). Differences in mean values of lipid peroxide or number of chewable foods between the four groups were assessed by analysis of variance with the Bonferroni-Dunn correction. The software package used for computer-assisted statistical analysis was StatView 5.0 (SAS institute, Cary, NC). Results were considered statistically significant when the p value was less than 0.05, while p below 0.0083 was considered to indicate significance with the Bonferroni-Dunn correction.

Results

Table 1 shows physical findings including number of teeth, clinical laboratory parameters, and other characteristics in the 85 year-old population. The mean number of teeth was 7.4±8.5. Mean concentrations of albumin, total cholesterol, triglyceride, HDL-cholesterol, hemoglobin (Hb) A1c, and lipid peroxide were all within the normal range. However, some differences were apparent between male and female subjects (numerical data not shown). Teeth were more numerous in men than that in women ($p = 0.0021$). Albumin and lipid concentrations in women were significantly higher than in men (albumin, $p = 0.0005$; total cholesterol, $p < 0.0001$; triglyceride, $p = 0.0115$; HDL-cholesterol, $p < 0.0001$), although no gender difference was noted for lipid peroxide. Smoking and alcohol consumption was more prevalent in male subjects (smoking, $p = 0.0002$; alcohol, $p < 0.0001$).

As shown in Table 2, Spearman’s rank correlation for number of teeth exhibited significant correlations with height (ρ= 0.215, $p = 0.0022$), white blood cell count (ρ= 0.223, $p = 0.0015$), and lipid peroxide (ρ= -0.200, $p = 0.0045$). We then adjusted for various confounding factors in a multiple regression analysis for lipid peroxide. Table 3 shows that number of teeth (β= -0.239, $p = 0.0018$), and total cholesterol (β= 0.267, $p = 0.0005$) remained significantly related to lipid peroxide.

In addition, by analysis of variance, we compared the lipid peroxide between four groups defined by number of teeth (Table 4). Mean lipid peroxide progressively increased from 2.26±0.64 nmol/ml in the group with at least 20 teeth to 2.50±0.66 nmol/ml in the group with 10 to 19 teeth, 2.73±0.83 nmol/ml in the group with 1 to 9 teeth, and 2.76±0.77 nmol/ml in the edentulous group (test for trend, $p = 0.0121$). Marked significance was noted between the group with at least 20 teeth and the edentulous group ($p = 0.0030$).

Finally, we compared denture status, Eichner Index, and number of chewable foods between four groups defined by number of teeth. Edentulous subjects and those with 1 to 9 teeth nearly all wore dentures (99% in each group).

Table 1. Characteristics of the 204 (n=204, 88 males and 116 females) 85-year-old subjects

	Mean	*S.D.*		*Number*	*%*
Height (cm)	149.1	9.8	Diabetes Mellitus	18	8.8
Weight (kg)	50.7	9.9	Hypertension	151	74.0
BMI, kg/m^2	22.7	3.5	Hyperlipidemia	82	40.2
Systolic blood pressure, mm Hg	144.0	24.4	Smoking	10	4.9
Number of teeth	7.4	8.5	Drinking	62	30.4
Albumin, g/dl	4.3	0.3	Exercise	140	68.6
Education, years	9.5	2.5	Dental care	44	21.6
Total cholesterol, mg/dl	194.3	37.0			
Triglyceride, mg/dl	122.7	55.9			
HDL-cholesterol, mg/dl	51.5	13.4			
Lipid peroxide, nmol/ml	2.63	0.76			
HbAlc, %	5.5	0.7			

Table 2. Speaman's rank correlation for number of teeth

	ñ	*P*
Height	0.215	0.0022
Weight, kg	0.128	0.0687
BMI, kg/m^2	-0.027	0.7041
Systolic blood pressure, mm Hg	0.054	0.4421
Albumin, g/dl	-0.032	0.6527
Total cholesterol, mg/dl	-0.037	0.6026
Triglyceride, mg/dl	0.008	0.9106
HDL-cholesterol, mg/dl	0.054	0.4447
Lipid peroxide, nmol/ml	-0.200	0.0045
HbA1c, %	0.025	0.7245
White blood cells, I	0.223	0.0015
Red blood x 10^4/I	-0.027	0.7048

Table 3. Multiple regression analysis for lipid peroxide

	â	*P*
Number of teeth	-0.239	0.0018
Gender	-0.098	0.2407
BMI, kg/m2	0.090	0.2186
Systolic blood pressure, mm Hg	0.024	0.7388
Total cholesterol, mg/dl	0.267	0.0005
HbA1c, %	-0.047	0.5237
White blood cells, I	0.075	0.3380
Smoking	-0.001	0.9883
Drinking	0.051	0.5041
Exercise	0.053	0.4587
Dental care	0.084	0.2466

Table 4. Analysis of variance with Bonferroni-Dunn correction of lipid peroxide number of chewable foods (*p<0.0083 is significant after the Bonferroni-Dunn correction)

Number of teeth	*Lipid peroxide, nmol/ml*		*Number of chewable foods*	
	mean ±	*p vs. ≥ 20-teeth*	*mean ±*	*p vs. ≥ 20-teeth*
≥20	2.26 ± 0.64	-	13.4 ± 2.3	-
10-19	2.50 ± 0.66	0.1792	11.4 ± 3.5	0.0280
1-9	2.73 ± 0.83	0.0098	10.0 ± 4.2	0.0002
0	2.76 ± 0.77	0.0030	9.6 ± 4.3	<0.0001

In both of these groups, all subjects were in the Eichner Index A3 class (100%), resembling the group with at least 20 teeth (100% being in the A3 class) and the group with 10 to 19 teeth (90% being in the A3 class). Thus, denture use appeared to eliminate differences in masticatory ability between the four groups. However, analysis of variance showed numbers of chewable foods to differ significantly between the four groups defined by number of teeth (Table 4). Mean number of chewable foods progressively decreased from 13.4±2.3 in the group with at least 20 teeth to 11.4±3.5 in the group with 10 to 19 teeth, 10.0±4.2 in the group with 1 to 9 teeth, and 9.6±4.3 in the edentulous group (test for trend, $p < 0.0001$). Marked significance was noted for the difference between the group with at least 20 teeth and the edentulous group using the Bonferroni-Dunn correction ($p < 0.0001$). Since number of chewable foods and lipid peroxide concentrations each had a significant, but reverse relationship to number of teeth, we investigated the relationship between number of chewable foods and lipid peroxide concentrations. Spearman's rank correlation between them approached significance ($\rho = -0.123$, $p = 0.0817$).

Discussion

The major finding in the present study in an 85-year-old population was that after adjustment for confounding factors, serum lipid peroxide was negatively associated with number of teeth. This is the first report to suggest a relationship at that age between dental variables such as number of teeth and oxidative stress as represented by lipid peroxide.

Lipid peroxide is a generic term for lipids produced by auto-oxidation in the presence of reactive oxygen species (ROS) and polyunsaturated fatty acids. Lipid peroxide has been measured as an index of overall oxidative stress using various methods such as assays for TBARS. Oxidative stress presently is considered to be a major cause of atherosclerosis in general and cardiovascular disease in particular (Halliwell, 1994). Many reports have documented the elevation of lipid peroxide in patients with atherosclerotic complications such as diabetic angiopathy (Yagi, 1987) and ischemic heart disease (Stringer *et al*., 1989). In addition, some interventional experiments have supported such associations. Increasing lipid peroxide in the blood of rabbits by injecting it into an ear vein produced endothelial abnormalities in the thoracic aorta (Yagi, 1987). Also, in patients with acute myocardial infarction, administration of antioxidant vitamins not only reduced lipid peroxide but also decreased risk of further myocardial necrosis and certain complications in comparison with a placebo group (Singh *et al*., 1996). Also, even in persons aged 80 and older, including our present subjects, baseline plasma concentrations of lipid peroxide predicted the risk of future cardiovascular events. For example, subjects in the highest quartile for fluorescent products of lipid peroxidation (FPLPs) had a cardiovascular risk seven times greater than those in the lowest quartile (Mezzetti *et al*., 2001). Like excessive FPLPs, elevated TBARS in the blood indicates greater oxidative stress likely to be harmful for vessels, ultimately resulting in vascular disease. The elevation of lipid peroxide accompanying tooth loss in the present study may convey increased risk of atherosclerosis even in very old persons. We are planning further follow-up of our 85-year-old subjects.

Since the present study was cross-sectional in nature, we could not isolate the reason why lipid peroxide was negatively associated with number of teeth. However, certain factors could influence both variables. For example, poor dietary habits relevant to dental and general health, such as lifelong consumption of a sugar-rich or fat-laden diet, may have caused both loss of teeth and higher lipid peroxide concentrations. Excessive daily intake of sweet or fatty foods leads to elevated serum lipid concentrations, which increase lipid peroxidation. As another aspect of the impact of diet and nutrition on oral diseases, sugars unequivocally represent the main etiologic factor for dental caries, which eventually lead to tooth loss and consequent impaired chewing ability, ultimately resulting in avoidance of hard and fibrous foods including fruits, vegetables, and whole grains (Moynihan, 2005). Very low intake (below 12 g/d) of non-starch polysaccharides (NSP), fruit, and vegetables also have been found in edentulous subjects (Moynihan, 2005). Poor dietary habits maintained over many years, such as a sugar-rich or fat-laden diet, may accelerate tooth loss and also increase serum lipid peroxides. As tooth loss impairs chewing ability, it can result in increased lipid peroxides in the blood via increased sugar and fat intake and decreased intake of antioxidant-rich foods such as fruits and vegetables. Indeed, edentulous subjects 65 or more old were reported to have significantly less intake of vitamin C than subjects of similar age who retained teeth (Sheiham *et al*., 2001); in another study, number of teeth correlated negatively with serum triglycerides concentration (Norlen *et al*., 1996). In the present study, we lack data concerning food intake, which precludes evaluation of the relationship between food intake and serum lipid peroxides, number of teeth, or masticatory ability. However, we found that although even the edentulous group had a good occlusion status according to the Eichner Index while wearing dentures, the number of foods considered chewable was much lower than in the group with at least 20 teeth; this could shift dietary intake toward sugars and fats at the expense of antioxidant-rich foods. We could not find a conclusively significant relationship between number of chewable foods and lipid peroxides in serum ($\rho = -0.123$, $p = 0.0817$), but, this correlation might have fallen short of significance because of denture use. Further study is required to explain why lipid peroxide concentration was negatively associated with number of teeth.

Last, a limitation of tooth loss data in elderly persons should be mentioned, specifically that loss reflects not only various diseases including caries and periodontal disease, but also nondisease factors such as cost of dental treatment and varying patient and dentist preferences concerning tooth preservation. Thus, an apparent relationship of number of teeth to biologic markers might not represent a true biologic link. A longitudinal study would be better suited to determine how dental status including tooth loss influences general health, which is expressed by biologic markers.

In conclusion, we found in an 85-year-old population that number of teeth was negatively associated with serum lipid peroxide concentration, which presumably reflects oxidative stress. This suggests that when more teeth remain less oxidative stress accumulates, which may decrease development and progression of atherosclerosis, since increased oxidative stress is a risk factor. Although we do not yet know whether this finding is applicable to all ages, increased oxdative stress could contribute to the association between tooth loss and atherosclerotic disease in the elderly.

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