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Physical Function Is Weakly Associated with Angiotensin-Converting Enzyme Gene I/D Polymorphism in Elderly Japanese Subjects

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Key Words
Physical function · Angiotensin-converting enzyme · Gene polymorphism · Elderly

Abstract
Background: The turning point in the deterioration of physical function seems to occur between the ages of 70 and 80 years. In particular, muscle strength may decline even more in subjects older than 75. A recent study found that the angiotensin-converting enzyme (ACE) genotype also affects physiological left ventricular hypertrophy. A very limited number of papers have examined genetic differences in resistance and endurance forms of a single sporting discipline. ***Objective:*** The purpose of this study was to evaluate the relationship between ACE genotype and physical function by controlling the known confounding factors including dental status. ***Methods:*** We selected 431 subjects who were aged 76 years and did not require special care for their daily activities. We conducted a medical examination, followed by 5 physical function tests, as follows: (1) maximum hand grip strength, (2) maximal isometric knee extensor strength, (3) maximal stepping rate for 10 s, (4) one-leg standing time with eyes open and (5) 10-meter maximum walking speed. Subjects were genotyped for the ACE intron 16 Alu insertion. In addition, serum concentrations of total cholesterol, total protein, IgA and IgG were measured at a commercial laboratory. The Eichner index was used as an indicator of occlusal condition. Multiple linear regression analysis was performed to evaluate the relationship between the ACE gene insertion/deletion (I/D) polymorphism and physical function considering confounding factors. ***Results:*** The ACE gene I/D polymorphism was positively associated with hand grip strength and 10-meter maximum walking speed. Betas of hand grip strength were 0.09 for I/D ($p = 0.022$) and 0.12 for insertion/insertion (I/I; $p = 0.004$). Betas of 10-meter walking speed were −0.11 for I/D ($p = 0.093$) and −0.14 for I/I ($p = 0.039$). Dental status such as Eichner index class C was sig-

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nificantly associated with one-leg standing time with eyes open (beta −0.11; p = 0.028). ***Conclusion:*** This study suggests that there is a significant relationship between ACE genotype and physical function. In particular, subjects with the ACE deletion/deletion genotype were associated with upper extremities.

Introduction

Generally, age-related physical disability and deterioration of physical function are becoming priorities in public health. Many functions of the human body decrease more rapidly in the period between the ages of 70 and 80 years. The turning point in the reduction of physical function also seems to occur in this period. In particular, muscle strength may decline even more in subjects older than 75 [1, 2].

A polymorphic insertion/deletion (I/D) variation in intron 16 of the angiotensin-converting enzyme (ACE) gene locus was identified over 15 years ago [3]. Individuals with the ACE deletion/deletion (D/D) genotype have higher plasma, cardiac tissue and lymphocyte ACE levels than ACE insertion/insertion (I/I) carriers [4, 5]. A recent study suggested that the ACE genotype also affects physiological left ventricular hypertrophy [6].

A very limited number of papers [7, 8] have examined genetic differences in resistance and endurance forms of a single sporting discipline. The relationship between the D allele of the ACE I/D polymorphism and power sports undertaken over a short time period is distinct from that of the I allele and enhanced endurance performance [9]. The I allele of ACE was associated with increased type I skeletal muscle fibers, which may be a mechanism for the association between the ACE genotype and endurance performance [10]. These studies show that the ACE gene I/D polymorphism is an influential genetic factor in physical performance.

In spite of these findings, the majority of the studies conducted to date have had a relatively small sample size and have included groups of young subjects or athletes. A significant relationship has been difficult to establish, especially for the elderly, as results are easily confounded by other factors such as gender, physique and dental status, among others [11].

The purpose of this study was to evaluate the relationship between ACE genotype and physical function by controlling the known confounding factors including dental status.

Methods

Study Population

The population for this study was drawn from the Niigata study. Briefly, the Niigata study was a prospective community-based study that was initiated in 1998 to evaluate the relationship between individuals' general health status and their history of dental diseases. Initially, questionnaires were sent to all inhabitants of Niigata City, Japan, aged 70 years (n = 4,542), based on a registry of residents in the city; all recipients were informed of the purpose of this survey. Among those who were randomly selected to participate in the Niigata study (n = 600), 431 subjects (228 males and 203 females) who turned 70 in 1998 and were aged 76 in 2004 underwent annual dental examinations. All subjects were Japanese, in good general health and did not require special care for their daily activities. The subjects who we selected from the study were homogenous in terms of race, and we restricted age to 76 years to exclude the influence of race and age variations on our results. The examination protocol that was used in the study was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Niigata University.

Clinical Assessments

We conducted a medical examination, followed by 5 physical function tests, as follows. (1) Maximum hand grip strength in both the dominant and nondominant hand was measured using a Smedley hand dynamometer (DM-100s, Yagami Inc., Nagoya, Japan). The score used was the best of the trials for both grip strengths. (2) Maximal isometric knee extensor strength was determined by a portable chair incorporating a strain gauge connected to a load cell. The subject sat on a seat in a vertical position that was adjusted so that he or she sat comfortably with legs hanging vertically and the knees bent at 90°. The test was performed twice on the right and left legs alternately. (3) Maximal stepping rate for 10 s was used as an index of agility using an industrial stepping rate counter (Stepping Counter, Yagami Inc.). The subject was instructed to step as fast as possible with each leg alternately while in a sitting position for 10 s. The stepping rate of the left and right legs was summed for this analysis. (4) One-leg standing time with eyes open was also measured. The static balance function was measured with the subject's eyes open and arms out, standing on one foot with the other off the floor. The score was either the number of seconds between when the non-preferred foot was raised and balance was lost (when the subject began to hop around or when the raised foot was lowered to the floor) or when 2 min had elapsed. The subjects performed one trial on each foot, and the best score was recorded. (5) We measured maximum walking speed by having the subjects walk at their fastest pace over a 10-meter course. The fastest walk was used.

Subjects were genotyped for the ACE intron 16 Alu insertion. Blood for leukocyte isolation and subsequent DNA preparation was collected in ethylenediaminetetraacetic acid-containing tubes at a final concentration of 50 mmol/l. Genomic DNA was amplified as previously described using polymerase chain reaction (PCR) with primers flanking the polymorphic region [12, 13]. PCR products of 490 and 290 bp were separated on 1.5% agarose gels and visualized by ethidium bromide staining. Because the D allele in heterozygous samples is preferentially amplified, each sample that was found to have the D/D genotype was sub-

Table 1. Comparison of selected characteristics between male and female subjects

Variable	Males (n = 228)	Females (n = 203)	p value
ACE gene I/D polymorphism			
D/D	26	27	
I/D	85	84	
I/I	88	64	0.319
Number of present teeth	16.3 ± 9.9 (226)	15.7 ± 9.5 (195)	0.498
Eichner index			
Class A	55	41	
Class B	103	92	
Class C	68	62	0.719
Hand grip strength, kg	36.2 ± 5.7 (215)	23.8 ± 4.2 (176)	<0.001
Leg extension strength, kg	35.8 ± 10.3 (188)	24.6 ± 7.5 (144)	<0.001
Stepping rate	83.7 ± 15.6 (197)	75.7 ± 13.3 (158)	<0.001
One-leg standing time with eyes open, s	54.8 ± 43.2 (199)	35.3 ± 36.3 (166)	<0.001
10-meter walking speed, s	4.6 ± 1.2 (195)	5.2 ± 1.0 (160)	<0.001
BMI	22.4 ± 2.8 (219)	23.3 ± 3.3 (191)	0.004
High blood pressure, mm Hg	134.0 ± 18.5 (221)	130.7 ± 15.8 (191)	0.051
Low blood pressure, mm Hg	76.5 ± 12.4 (221)	72.2 ± 10.2 (191)	<0.001
Number of medications	4.4 ± 2.9 (125)	3.9 ± 3.0 (114)	0.213
Patient's feeling of health			
Excellent	12	10	
Good	153	141	
Not good	39	29	
Bad	15	8	0.595
Total cholesterol, mg/dl	187.3 ± 28.9 (218)	206.9 ± 30.0 (187)	<0.001
Total protein, g/dl	6.9 ± 0.5 (218)	7.0 ± 0.5 (187)	0.205
IgA, mg/dl	284.1 ± 129.4 (218)	246.1 ± 86.9 (187)	0.001
IgG, mg/dl	1,284.7 ± 264.6 (218)	1,310.5 ± 285.8 (187)	0.346

Figures represent number of patients, except where indicated otherwise. Figures in parentheses show the number of patients evaluated for the relevant characteristic.

jected to a second, independent PCR amplification with a primer pair that recognizes an insertion-specific sequence to avoid mistyping the D/D genotype. In addition, serum concentrations of total cholesterol, total protein, IgA and IgG were measured at a commercial laboratory (BML Inc., Tokyo, Japan).

The Eichner index [14] was used as an indicator of occlusal condition. The Eichner index is based on existing natural tooth contacts between the maxilla and mandible in the bilateral premolar and molar regions (existence of tooth contact defined as existence of natural tooth in the maxilla and mandible correspondingly). Class A represents contact in all 4 support zones. Class B represents contact in 1–3 zones or in the frontal region only. Class C represents an absence of tooth contact.

Statistical Analysis

All continuous variables were checked for normality. One-leg standing time with eyes open was skewed to higher values and was transformed to dummy data when conducting the statistical tests. Multiple linear regression analysis was performed to evaluate the relationship between ACE gene I/D polymorphism and physical function. In the model, each physical function variable was used as a dependent variable. Independent variables included serum data such as total cholesterol, total protein, IgA and IgG, dental status (Eichner index class C = 1, no = 0), ACE gene I/D polymorphism, BMI and gender.

All calculations and statistical analyses were performed using the STATA™ software package (StataCorp, College Station, Tex., USA). A p value less than 0.05 was considered statistically significant.

Results

Characteristics of the subjects are shown in table 1. All physical function variables were better in males than in females. Results of multiple regression analysis of the relationship between the ACE gene I/D polymorphism and physical function are shown in table 2. The ACE gene I/D polymorphism was positively associated with hand grip strength and 10-meter maximum walking speed. Betas of

Table 2. Relationship between ACE gene I/D polymorphism and physical function after adjustment for confounding factors using multiple regression analysis

Independent variables	Hand grip strength		Leg extension strength		Stepping rate		One-leg standing time with eyes open		10-meter walking speed	
	beta	p value	beta	p value	beta	p value	beta	p value	beta	p value
Total cholesterol	−0.05	0.148	0.01	0.912	0.03	0.596	0.05	0.422	−0.08	0.205
Total protein	0.01	0.776	0.04	0.581	−0.03	0.695	−0.02	0.809	−0.04	0.547
IgA	−0.05	0.126	0.00	0.922	0.07	0.221	0.03	0.553	0.03	0.634
IgG	−0.04	0.354	−0.10	0.100	−0.03	0.648	−0.03	0.670	0.07	0.318
Dental status[a]	0.01	0.687	0.02	0.669	−0.04	0.458	−0.11	0.028	0.09	0.073
ACE gene I/D polymorphism										
D/D[b]	0.00	–	0.00	–	0.00	–	0.00	–	0.00	–
I/D	0.09	0.022	0.09	0.143	0.13	0.042	0.07	0.250	−0.11	0.093
I/I	0.12	0.004	0.08	0.177	0.03	0.610	0.08	0.209	−0.14	0.039
BMI	0.10	0.001	0.22	<0.001	−0.03	0.622	−0.15	0.002	0.04	0.419
Gender[c]	−0.78	<0.001	−0.55	<0.001	−0.26	<0.001	−0.13	0.014	0.25	<0.001
Constant	–	<0.001	–	0.042	–	<0.001	–	0.037	–	<0.001
n	382		325		347		403		347	
R^2	0.63		0.33		0.09		0.07		0.10	

[a] Eichner index class C = 1, no = 0. [b] Reference; beta = 0. [c] Male = 0, female = 1.

hand grip strength were 0.09 for I/D (p = 0.022) and 0.12 for I/I (p = 0.004). Betas of 10-meter maximum walk were −0.11 for I/D (p = 0.093) and −0.14 for I/I (p = 0.039). Dental status such as Eichner index class C was significantly associated with one-leg standing time with eyes open (beta −0.11; p = 0.028).

Discussion

In aged societies, enhancement of quality of life has been regarded as more crucial than prolongation of life expectancy. It is well known that people with impaired activities of daily living, such as walking, eating and toileting, or with low instrumental activities of daily living tend to have a high mortality rate [15]. We adopted hand grip strength, leg extensor strength, stepping, one-leg standing time with eyes open and 10-meter maximum walking speed as indices of physical function. Physical function can be classified roughly into muscle strength, agility and equilibrium function. In the present study, the ACE gene I/D polymorphism was positively associated with hand grip strength and 10-meter maximum walking speed. However, this finding did not demonstrate an association with muscle strength, because the results showed that only hand grip strength was significantly associated with the ACE gene I/D polymorphism.

Indeed, the results showed that leg extensor strength was not associated with the ACE gene I/D polymorphism. On the other hand, the ACE gene I/D polymorphism was positively associated with 10-meter maximum walking speed, which evaluated the lower extremities. The ACE genotype might appear to have at best a minor influence on muscle size in the lower extremities. The reason for the difference in the association with ACE genotype between the upper and lower extremities was unclear.

According to previous reports, ACE inhibitor treatment may halt or slow decline in muscle strength in elderly women with hypertension and without congestive heart failure [16]. Improvements in physical function from ACE inhibitors and angiotensin receptor antagonists could also be mediated by direct effects of these agents on skeletal muscle [17, 18]. In particular, activation of the renin-angiotensin system has been associated with mechanical, metabolic and biochemical changes in skeletal muscle [19]. Furthermore, the D/D genotype has been found to be associated with increased cardiovascular disease [20]. Because the ACE gene is involved in regulating vascular tone, the finding that the ACE I/I genotype group has a wider maximal arteriovenous O_2 difference suggests a greater release of peripheral vascular tone with an attendant greater increase in capillary perfusion and red cell transit time in the ACE I/I than in the I/D and D/D genotype groups [21]. On the other hand,

Frederiksen et al. [22, 23] evaluated an association between ACE genotype and physical and cognitive performance, and they found no significant effects of ACE genotype on hand grip strength in spite of a similar trend of findings as that of our study [23]. The cause of the discrepancy in our study was unclear. A possible explanation could be that the subjects in the studies of Frederiksen et al. [22, 23] had a wide range of ages. We selected subjects of the same age to exclude the influence of age variation on the results. A significant relationship between ACE genotype and physical performance has been difficult to establish, as results are easily confounded by other factors such as nutritional deficiencies, smoking, immune dysfunction and bone mineral density, among others.

In addition, we found a significant relationship between dental status, such as occlusal condition, and physical function, especially one-leg standing time with eyes open, after controlling confounding factors. One-leg standing time with eyes open is an index of equilibrium function. Several investigators have reported the influence of oral status on motor performance and muscle strength of the lower extremities [24, 25]. Some relation between occlusion and posture regulation has been reported [26–28]. Abnormal habituation such as one-sided mastication may be precipitated by a change in occlusal support. Subsequently, abnormal habituations may lead to a disequilibrium of systemic muscle balance and may have some influence on systemic equilibrium function.

One limitation of our study is that we could not confirm a clear cause-and-effect relationship among ACE genotypes, occlusal condition and physical function in the elderly because of our cross-sectional design. In order to explore the actual relationship, further prospective studies and clinical trials which consider confounding factors, including mortality, will be necessary.

In conclusion, this study suggests that there is a significant relationship between ACE genotype and physical function. In particular, subjects with the ACE D/D genotype were associated with upper extremities.

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Effects of dental treatment on the quality of life and activities of daily living in institutionalized elderly in Japan

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ABSTRACT

Impairment of oral health has a negative impact on the quality of life (QOL) of the elderly. Activities of daily living (ADL) are known to be an important determinant of their QOL. A controlled study was conducted between September and November 2007 to determine the effects of dental treatments on the QOL and ADL among 30 institutionalized Japanese elderly who were allocated into two groups, an intervention group and a control group. Their mean age was 80 ± 9 years. Outcome data were collected 6 weeks after baseline in both groups. QOL and ADL were assessed using General Oral Health Assessment Index (GOHAI) and Functional Independence Measure (FIM). The intervention group, which had received dental treatment, showed significant increases in GOHAI scores between baseline and 6 weeks ($p = 0.04$), whereas no significant difference was found between baseline and 6 weeks in the control group. The differences in the changes in the FIM scores for expression were significant in the model adjusted for covariables ($p = 0.03$). Our findings showed that dental treatments increased the oral health-related QOL and the expression function in the ADL. Promoting dental care service at nursing facilities may be beneficial for maintaining the residents' QOL.

1. Introduction

Even faster than in most Western and Asian countries, Japanese society is aging. People 65 years and older constituted 20.1% of the Japanese population according to the 2005 census (http://www.stat.go.jp/data/kokusei/2005/index.htm). Maintaining good health and quality of life (QOL) is essential for the benefit of both individuals and society. Moreover, a growing number of elderly people are opting to spend the last years of their lives in long-term care facilities (Grbich et al., 2005; Hirakawa et al., 2007).

Oral health care in long-term facilities, however, has repeatedly been documented as being less than ideal (Sweeney et al., 2007; Unlüer et al., 2007). Two studies have reported the effects of oral care on health status in nursing home elderly. Yoneyama et al. (2002) reported that improving oral hygiene could lower the risk of respiratory infections, while Watando et al. (2004) suggested that intensive oral care reduced the incidence of pneumonia by improving the cough reflex sensitivity in those who were physically disabled or suffering from mental deterioration.

Yoshino et al. (2001) showed an improvement in the activities of daily living (ADL) with intensive oral care among elderly nursing home patients with dysphagia due to cerebrovascular disease. Their finding suggested that oral care reduced the risk of pneumonia by improving the swallowing reflex and overall functional status. Eating begins from the anticipatory stage and proceeds through preparatory, lingual, pharyngeal, and esophageal stages (Leopold and Kagel, 1983). Maintaining oral function with dental treatments also might improve ADL, but the effects of dental treatments on the ADL in the elderly have not been sufficiently examined.

Impairment of oral health has a negative impact on the QOL of the elderly (Mariño et al., 2008). Some studies have found that ADL are an important determinant of the QOL of elderly nursing home residents (Tseng and Wang, 2001; Tu et al., 2006). Therefore, we focused on this relationship and conducted a controlled study to determine whether dental treatments affect the QOL and ADL in institutionalized elderly.

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2. Materials and methods

2.1. Subjects and study design

This was a controlled study conducted at seven nursing homes in Japan between September and November 2007. The study consisted of three phases: subject screening and enrollment, and allocation into intervention and control groups; baseline assessments of all subjects; an intervention phase in which the intervention group received dental treatment. Outcome data were collected 6 weeks after baseline.

Those who met the following two criteria were eligible for participation: residence in the facility for at least 30 days and an age of 65 years or above. Written informed consent was obtained from all patients. The staff in the research administration office allocated the subjects into two groups in terms of their gender, age, and independence levels. This study was approved by the Ethics Review Committee of Fujita Health University School of Medicine in September 2007.

2.2. Data collection

ADL and QOL were assessed using the Functional Independence Measure (FIM) and General Oral Health Assessment Index (GOHAI). The FIM is an 18-item, 7-level ordinal scale that measures a patient's ability to perform common daily tasks (Granger et al., 1986). Of these, four items were assessed for this study: feeding, dressing, transfer, and expression. The FIM scores were determined by the doctors in the nursing facilities.

The GOHAI is an oral health-related QOL instrument that was originally developed in the United States for use among the elderly (Atchison and Dolan, 1990). Other language versions of the GOHAI as well as Japanese (Naito et al., 2006) have been developed and widely used. It suggests that health-related QOL be defined as a person's assessment of how the following affect his or her well-being: functional factors, psychological factors, social factors, and the experience of pain/discomfort. When these considerations center on orofacial concerns, oral health-related QOL is assessed (Ingelehart and Bagramian, 2002). The GOHAI contains 12 questions. Each question has a score between 1 and 5, and the total score for the 12 questions is the GOHAI score (maximum 60, minimum 12). One of its strengths is its ability to reveal the psychosocial impact of oral health. The GOHAI data were collected at dentist interviews.

Information regarding daily life, such as diet and oral health behavior, were provided by the care staff at the facilities. Dentists in the research group assessed oral health status in subjects at baseline and treated them according to the study protocol.

2.3. Statistical analysis

The differences in the demographic characteristics of the two groups at baseline were analyzed using the χ^2-test for proportions and t-test for continuous variables. Two measurements in the GOHAI and FIM scores were compared using Pearson's correlation.

The changes in the GOHAI and FIM scores between baseline and 6 weeks were analyzed using paired t-test. Analysis of covariance (ANCOVA) was first adjusted for gender and age between the two groups (Model 1). Then, a second model (Model 2) added other confounding factors for statistical adjustment.

All p values were two-sided, and statistical significance was set at 5%. All the analyses were performed using SPSS for Windows, version 14.0 (Statistical Product and Service Solutions, Chicago, IL, USA).

3. Results

In September 2007, we allocated 30 subjects (12 men and 18 women) to the study, 15 each to the intervention and control groups, respectively. Five withdrawals occurred, three in the intervention group and two in the control group. Of the withdrawals in the intervention group, one rejected the dental treatments and two had to discontinue treatments because their health worsened. In the control group, one rejected the dental treatments and the other died within 6 weeks.

One subject in the intervention group needed to be hospitalized for dental treatments and the intervention was delayed 6 weeks due to bed unavailability. Therefore, this subject was analyzed as a member of the control group. The analysis included 11 in the intervention group and 14 in the control group. Consequently, the number of subjects classified by treatment in each group was as follows: in the intervention group, 8 required dentures to be made or repaired, 6 required endodontic or periodontal treatment, and 6 required oral care; in the control group, 10 required dentures to be made or repaired, 10 required endodontic or periodontal treatment, and 9 required oral care. The mean treatment duration was 5.0 ± 1.8 weeks in the intervention group.

Table 1 shows the baseline characteristics of the two groups. The mean age for all subjects was 80 ± 9 years (range 66–97). The control group had a lower body mass index (BMI) than the intervention group ($p = 0.01$). All of the subjects took their food orally, but a greater proportion of the subjects in the intervention group needed an adjustment of their daily diet (100% vs. 57%, $p = 0.01$). The other baseline characteristics of the two groups were similar.

The baseline GOHAI scores did not differ significantly between the two groups. In Table 2, the intervention group showed significant increases in the GOHAI scores between baseline and 6 weeks ($p = 0.04$), while no significant difference was observed in

Table 1
Baseline characteristics of study participants in two groups, mean ± S.D. or n (%).

	Intervention ($n = 11$)	Control ($n = 14$)	p^*
Age (year)	78.2 ± 9.9	81.2 ± 7.9	0.40
Women	7 (63.6)	8 (57.1)	0.74
BMI	23.7 ± 3.8	20.2 ± 2.2	0.01
Chronic conditions	0.6 ± 0.7	0.8 ± 0.9	0.65
Duration of institutionalization, y	7.8 ± 9.4	3.8 ± 4.9	0.21
Mental health GHQ ≥ 4, %	44.4	53.8	0.66
Independence levels			
Normal	0 (0.0)	1 (7.1)	
J	1 (9.1)	1 (7.1)	
A	3 (27.3)	6 (42.9)	0.78
B	6 (54.5)	5 (35.7)	
C	1 (9.1)	1 (7.1)	
Dietary intake,			
Oral intake without any adjustments	0 (0.0)	6 (42.9)	0.01
Oral intake with adjustments	11 (100.0)	8 (57.1)	
Tooth loss	17.3 ± 4.4	17.0 ± 7.4	0.92
Tooth decay	1.7 ± 2.3	1.7 ± 2.7	0.99
Wear dentures	7 (63.6)	9 (64.3)	0.97

* χ^2-test for proportions and t-test for continuous variables.

Table 2
Changes in GOHAI scores between baseline and 6 weeks in two groups.

Group	GOHAI score, mean ± S.D.		
	Baseline	6 weeks	p^*
Intervention ($n = 11$)	47.9 ± 9.7	54.2 ± 7.3	0.04
Control ($n = 14$)	49.7 ± 9.8	50.9 ± 7.9	0.45

* Paired t-test.

Table 3
Changes in GOHAI scores between baseline and 6 weeks in two groups.

Group	Model 1[a]				Model 2[a]			
	Mean[b]	S.E.	Difference (95% CI)	p	Mean[c]	S.E.	Difference (95% CI)	p
Intervention (n = 11)	6.8	1.9	6.1 (0.6–11.5)	0.03	8.5	2.0	9.1 (2.9–15.2)	0.006
Control (n = 14)	0.7	1.7			−0.6	1.8		

[a] Adjusted increase in FIM scores between baseline and 6 weeks.
[b] Adjusted for gender and age.
[c] Adjusted by gender, age, dietary intake pattern, and facilities.

Table 4
Changes in FIM scores between baseline and 6 weeks in two groups.

FIM items	Intervention (n = 11)			Control (n = 14)		
	Baseline	6 weeks	p^*	Baseline	6 weeks	p^*
Feeding, mean (S.D.)	5.8 (1.7)	5.9 (1.7)	0.34	6.2 (0.9)	6.2 (0.9)	–
Dressing, mean (S.D.)	3.0 (2.2)	3.1 (2.2)	0.34	5.3 (1.9)	5.0 (2.1)	0.26
Transfer, mean (S.D.)	3.0 (2.0)	3.5 (2.3)	0.14	4.6 (2.5)	4.4 (2.5)	0.34
Expression, mean (S.D.)	2.6 (2.3)	4.8 (2.1)	0.02	4.5 (2.6)	5.4 (1.7)	0.03

[*] Paired t-test.

Table 5
Changes in FIM scores for expression between baseline and 6 weeks in two groups.

Group	Model 1[a]				Model 2[a]			
	Mean[b]	S.E.	Difference (95% CI)	p	Mean[c]	S.E.	Difference (95% CI)	p
Intervention (n = 11)	2.2	0.7	1.3 (−0.6–3.1)	0.16	2.1	0.4	1.2 (0.1–2.3)	0.03
Control (n = 14)	0.9	0.6			1.0	0.3		

[a] Adjusted increase in FIM scores for expression between baseline and 6 weeks.
[b] Adjusted for gender and age.
[c] Adjusted for gender, age, frequency of private training, frequency of group training, frequency of recreational activities, independence level at baseline, and facilities.

the control group. The differences in the changes in the GOHAI scores for the two groups were significant ($p < 0.05$) in each model when adjusted for potential confounding variables, with or without inclusion of the dietary intake pattern and facilities; the intervention resulted in an increase in the GOHAI scores (Table 3).

The baseline scores for the FIM items except dressing ($p = 0.01$) did not differ significantly between the two groups. No significant increase in the scores for the FIM items except the expression occurred between baseline and 6 weeks in either group (Table 4). As shown in Table 5, the differences in the changes in the expression scores for the two groups were significant ($p = 0.03$) in the model adjusted for gender, age, frequency of private training, frequency of group training, frequency of recreational activities, independence level at baseline, and facilities; the intervention resulted in an increase in the expression scores. The differences in the changes in the dressing scores for the two groups were also detected in the ANCOVA ($p = 0.09$).

The GOHAI scores were related to the transfer and dressing scores for the FIM items at baseline ($p = 0.03$ and $p = 0.08$, respectively). The changes in the GOHAI scores between baseline and 6 weeks were associated with those of dressing and expression in FIM scores ($p = 0.049$ and $p = 0.08$, respectively).

4. Discussion

In this controlled, 6-week study, we found that the dental treatments for institutionalized elderly increased the oral health-related QOL and the expression function in the ADL. No significant beneficial effects on feeding or transfer function were found, while dressing function tended to increase. To our knowledge, this is the first controlled study involving nursing home residents to show that dental treatments influence the ADL and QOL.

With old age, increased dependency affects the QOL negatively. Arslantas et al. (2009) indicated that medicosocial services for elderly people needed to be prioritized to improve the QOL and health training. For elderly residents of nursing homes, in addition to receiving good care and maintaining a strong physical condition and a healthy mental state, preserving QOL is important (Kane et al., 2003). Zanocchi et al. (2008) reported that chronic pain in institutionalized elderly was common and worsens their QOL. In our study, as the importance of assessing and managing pain was emphasized, reducing chronic pain by dental treatments could help to improve the QOL in some cases.

The increase of expression scores in the intervention group was significantly higher than those in the control group, while these scores in both groups improved. The expression can be a sensitive marker of events in everyday life. The dental treatment likely restored the subject's oral function and led to the improved expression. Furthermore, conducting the intervention might have stimulated the subjects in their daily life. The difference between groups might have arisen from the ADL status just before the intervention. The expression was lower than other items in the intervention group at baseline. Other scores including feeding did not improve significantly. These results suggest the difficulty in detecting small changes in the ADL with intervention in elderly people who have maintained a certain level of independence.

The QOL increased significantly with the intervention. Dental treatments, such as making dentures, often need time to alter the patients' symptoms and allow them to recover from oral dysfunction. Therefore, studies using subjective indicators must follow to evaluate the effects of dental treatments. Feeding was not directly linked to the improvement in the QOL. Most of the dental treatments in this study could not be interventions that changed the subjects' eating function dramatically. In addition, all subjects

had maintained the pre-intervention level of oral intake, which might have contributed to the lack of a statistically significant change in the feeding scores.

The dental staffs can contribute to and facilitate improvement in nursing home residents' QOL by helping them maintain or regain optimum oral health and function, which contributes to their well-being and general health by furthering nutrition, alleviating pain and discomfort, and increasing personal esteem and social acceptability (Schembri and Fiske, 2005). Meanwhile, Pyle et al. (2005) indicated that oral health continued to have a low priority in nursing facilities. They also suggested that continuing efforts to improve oral health and educate long-term care professionals about the influence of oral health on general health was critical for managing the oral health of future generations of aging adults. Our findings provided further evidence that dental professionals need to recognize the importance of residential home care.

We targeted nursing home residents in this study. A previous study also revealed the impairment of oral health and oral health care needs for community-dwelling elderly people with disabilities (Saunders and Friedman, 2007). Further studies investigating the effects of dental treatments on ADL and QOL in various settings are needed. They will provide useful clues for future health care policy.

A few limitations to this study warrant consideration. First, the subjects were not allocated randomly into the two groups. However, the basic characteristics of the groups were similar and the analysis made statistical adjustments if necessary. Second, the dropout rate in this study was 17% (five subjects), although it did not differ significantly between the two groups. The reason for withdrawal of three of the five was worsening health, which could not be prevented. While one subject in the intervention group was analyzed in the control group, the intention-to-treat analysis showed that the results were the same as those for each protocol. In addition, the sample size was small and we could not conduct a stratified analysis of gender or other factors. Continuous efforts for further research are warranted to confirm these findings.

In conclusion, our findings indicated that dental treatments for the institutionalized Japanese elderly improved the oral health-related QOL and the expression function in the ADL. Promoting dental care service at nursing facilities may be beneficial for maintaining residents' QOL.

Conflict of interest

None.

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Original Article

Effects of IgY against *Candida albicans* and *Candida* spp. Adherence and Biofilm Formation

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SUMMARY: The fungal pathogen *Candida albicans* is an opportunistic fungal pathogen that causes oral and vaginal mucosal infections as well as systemic disease. The ability of *C. albicans* to adhere to host surfaces is positively correlated with its pathogenicity. We prepared a polyclonal anti-*Candida albicans* antibody in chicken egg yolk (anti-*C. albicans* IgY) and investigated its in vitro effectiveness in preventing *C. albicans* adherence and biofilm formation. Anti-*C. albicans* IgY significantly reduced the adherence of *C. albicans* SC5314 to human oral epithelial cells in a dose-dependent manner. The same effect was also observed in other *Candida* spp. including *C. albicans* serotype A and B. Further, the IgY inhibited biofilm formation of *C. albicans* in medium without serum, but the inhibition was slightly restored in medium conditioned with 10% serum. The data indicate that anti-*C. albicans* IgY cross-reacted with various *Candida* spp. and may have a protective effect against oral candidiasis and reduce the dissemination of *Candida* spp. This effect may be due to the blocking of the binding of *Candida* spp. to the host cells. However, the blocking did not play a role when *Candida* formed a germ tube in the presence of serum. Therefore, anti-*C. albicans* IgY may be considered as a prophylactic immunotherapy or possibly an adjunctive antifungal therapy under limited conditions.

INTRODUCTION

Most bacteria and fungi that exist in humans as surface-attached communities are called biofilms, and such communities usually affect human health. The tissues are the substrates for the formation of biofilms, and the microorganisms in the biofilms serve as reservoirs to continuously seed an infection. The fungal pathogen *Candida albicans* is an opportunistic fungal pathogen that causes oral and vaginal mucosal infections as well as systemic disease (1). The ability of *C. albicans* to adhere to host surfaces is positively correlated with its pathogenicity (2). It produces adherent biofilms on a variety of different surfaces in vitro (3-6). Biofilm formation begins with surface adherence of the yeast form, which grows to yield a basal layer. The basal layer cells include some hyphae, or long tubular chains of cells, which extend to yield an upper layer that is almost exclusively hyphae. As the biofilm matures, it produces an extracellular matrix containing predominantly carbohydrate and protein (7-9).

Adherence is a critical property for biofilm microbial cells, with multiple adhesion molecules functioning in successful biofilm formation. Specific adherence to the protein surface is provided by several surface adhesins of *Candida*. Recent reports have demonstrated that antibodies with defined specificities to these surface adhesins show different degrees of protection against systemic and mucosal candidiasis (10-12). Secretory immunoglobulin A (sIgA) is thought to play a central role by inhibiting *Candida* adherence to host cells (13-15). Complex mixtures of antibodies having different specificities such as those found in salivary sIgA are shown to decrease adhesion of *C. albicans* to the host surface but do not inhibit germination (16). Therefore, the use of antibodies as an adjunct to antifungal drugs may be considered one approach to protecting against candidiasis.

Chicken eggs are known as an inexpensive and convenient source for mass production of specific antibodies (17). Specific egg yolk immunoglobulin (IgY) can be produced in egg yolk by immunizing hens with specific antigens. IgY is isolated in large quantities from the yolk by simple methods without distress to the birds (18), and has been used extensively for the treatment and prevention of various infections in animals and humans with mixed success (19-26). In particular, polyclonal anti-*C. albicans* antibody in chicken egg yolk prevented *C. albicans* from adhering to oral epithelial cells where the effect depended on the density of the infection (27). However, the IgY was induced by immunization with the *C. albicans* yeast form and included antibodies against various antigens. In general, the activity and diversity of IgY against *Candida* spp. are not well understood. The objective of this study was to evaluate the efficacy of a specific IgY against *C. albicans* and other *Candida* strains to develop an alternative therapy for candidiasis.

MATERIALS AND METHODS

Yeast strains: *C. albicans* SC5314 (serotype A), NIH207 (serotype A) and NIH792 (serotype B), *Candida tropicalis* IFO0618, *Candida dubliniensis* CD36 and CD57, *Candida parapsilosis* ATCC22019 and FRCP-0201, *Candida glabrata*

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850821 and CBS138 were used. All strains were provided by Dr. Masakazu Niimi from the National Institute of Infectious Diseases. For use in experiments, all organisms were grown in liquid Yeast Peptone Dextrose (YPD; 2% Bacto peptone, 2% dextrose and 1% Yeast extract) broth aerobically at 37°C; and washed three times in phosphate-buffered saline (PBS). Then they were suspended to the appropriate concentration in PBS.

Preparation of IgY: Anti-*C. albicans* IgY was acquired by immunization of chickens with the yeast form, which was provided by GHEN Corporation (Tokyo, Japan) as a purified powder. A solution containing 4 mg/ml was prepared in PBS. Control IgY was prepared from the eggs of non-immunized hens. Fat-free egg yolk powder was purified for IgY using the ammonium sulfate precipitation method. The protein concentration was determined using the BioRad protein assay method (BioRad, Hercules, Calif., USA) based on the Bradford method. One milligram per milliliter of bovine serum albumin (IWAI, Tokyo, Japan) was used as the reference protein. The absorbance at 620 nm after a 30-min reaction with Bradford's solution was measured using a spectrophotometer.

Epithelial cells: The human oral squamous carcinoma cell lines, Ca9-22 and HSC-2, were purchased from the Japanese Collection of Research Bioresources in Health Science Research Resources Bank (Tokyo, Japan). They were maintained in Minimal Essential Medium Eagle's (SIGMA ALDRICH Corp., St. Louis, Mo., USA) containing 10% fetal bovine serum supplemented with 6 mg/ml L-glutamine, penicillin and streptomycin. They were grown on 24-well plates at 37°C in a humidified environment containing 5% CO_2 and used at 95% confluence in all experiments.

Antibody titration: Enzyme-linked immunosorbent assay (ELISA) was used to determine the titer of the specific antibody. Each well of a 96-well polystyrene plate was coated overnight at 4°C with 100 μl of whole yeast in PBS (OD_{660} = 1.0). The wells were washed with PBS-T (0.05% Tween 20 in PBS, PBS-T) and blocked with 150 μl 1.0% (w/v) skim milk in PBS-T for 1 h at 37°C. After three washes with PBS-T, various protein concentrations (0.032, 0.063, 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/ml) of IgY were added to the wells; and the plates were incubated for 1 h at 37°C. The plates were washed three times, and alkaline phosphatase-conjugated goat IgG polyclonal anti-chicken IgY (ABCAM PLC, Cambridge, UK) in PBS-T (1:5,000 dilution) was added. After five washes with PBS-T, bound antibodies were detected after adding 100 μl of 3 mg/ml para-nitrophenyl phosphate as a substrate and incubating for 60 min at 37°C. The optical densities were determined using a microplate reader (Multiskan Bichromatic Laboratory Japan, Tokyo, Japan) at 405 nm. The background (control) was defined in wells coated without IgY. All samples were tested in triplicate.

Effects of anti-*C. albicans* IgY on cell growth of *Candida* strains: Cell suspensions of *C. albicans* SC5314 and 0 or 2 mg/ml of anti-*C. albicans* IgY or 2 mg/ml control IgY were mixed and incubated in YPD or PBS for 24 h at 37°C in aerobic conditions. The absorbance at 660 nm was measured at 0, 1, 3, 6 and 24 h after incubation. To confirm visually the specificity of the anti-*C. albicans* IgY, 2 mg/ml of anti-*C. albicans* and 2 mg/ml of control IgY were applied to cell suspensions of *C. albicans* SC5314 cultivated in YPD or RPMI1640 with 10% fetal bovine serum (FBS); and incubated aerobically for 60 min at 37°C. The cells treated with anti-*C. albicans* IgY and control IgY were washed three times using sterile PBS and mixed with 1/1,000 diluted FITC-conjugated rabbit anti-chicken IgY antibodies (ANASPEC, Sun Jose, Calif., USA) for 60 min at 37°C. The cells were washed three times using sterile PBS and observed using a confocal laser scanning microscope (Olympas, Tokyo, Japan).

Effects of anti-*C. albicans* IgY on adherence of *Candida* strains: Absorbance at 660 nm was measured to adjust the yeast concentration to OD_{660} = 1.0. The yeast was mixed with 0.006, 0.0125, 0.25, 0.5, 1 or 2 mg/ml IgY and 2 mg/ml control IgY for 60 min at 37°C and added to the epithelial cells on a 24-well plate. After 60-min incubation, yeasts adhering to the epithelial cells were separated from free yeasts by washing three times with PBS. Then, 1 ml 0.05% trypsin-EDTA was added to each well, and the plates were incubated for 10 min at room temperature. The detached cell suspensions were collected in 0.5% trypsin-EDTA using the pipetting technique, and spread on the YPD agar plate using an EDDY JET spiral plating system (IUL, S.A., Barcelona, Spain). After incubation for 24 h at 37°C under aerobic conditions, the number of colonies on the plates was counted and compared to those on the plates that did not have IgY.

Effects of anti-*C. albicans* IgY on biofilm formation of *C. albicans*: Biofilm formation by *C. albicans* SC5314 was assayed using a method described previously (28,29), with some modification. *C. albicans* incubated for 24 h at 37°C in YPD broth was adjusted to OD = 0.5 at 660 nm, harvested by centrifugation and washed in PBS two times. The *C. albicans* suspension was diluted with RPMI1640, and 2 mg/ml anti-*C. albicans* IgY was added to the 96-well microtiter plate wells. The chemically defined RPMI1640 medium containing minimal (0.2%) glucose with or without 10% FBS was used as the nutrient-poor condition for the biofilm formation assay. After incubation for 24 h at 37°C, biofilms formed in wells were washed with sterile PBS two times. Biofilm formation was tested using the XTT assay at 492 nm. XTT reduction has been widely used to measure biofilm activity and allows the detection of small differences in metabolic activity between strains (30-32).

Statistical analysis: All data were analyzed using the Statistical Package for SPSS for Windows (version 100; SPSS, Chicago, Ill., USA). The Student's *t* test with the Bonferroni Method was used to compare data of treatment with control IgY and anti-*C. albicans* IgY. *P*-values less than 0.05 were considered to be significant.

RESULTS

Antibody titers of IgY to *Candida* spp. were measured using ELISA (Fig. 1). Anti-*C. albicans* IgY reacted to *C. albicans* SC5314, *C. tropicalis* IFO0618, *C. dubliniensis* CD36 and CD57 in a dose-dependent manner where the IgY titers were significantly elevated at concentrations of 0.032, 0.073 or 0.125 mg/ml increasing to 4 mg/ml of antibody (IgY). By contrast, the control IgY to these *Candida* spp. were poor in all tested concentrations, whereas 4 mg/ml of the control antibody reacted only slightly to *Candida* spp. The titers of both antibodies were similar to those of other *Candida* strains (data not shown). Two milligrams per milliliters of IgY showed a stronger response to *Candida* strains than the control IgY and was used in further experiments. Before the *Candida* adherence tests, the effect of IgY was tested to determine whether the antibodies inhibited cell growth of *C. albicans*. Anti-*C. albicans* IgY did not inhibit the cell growth of *C. albicans* in comparison to the cell growth in YPD

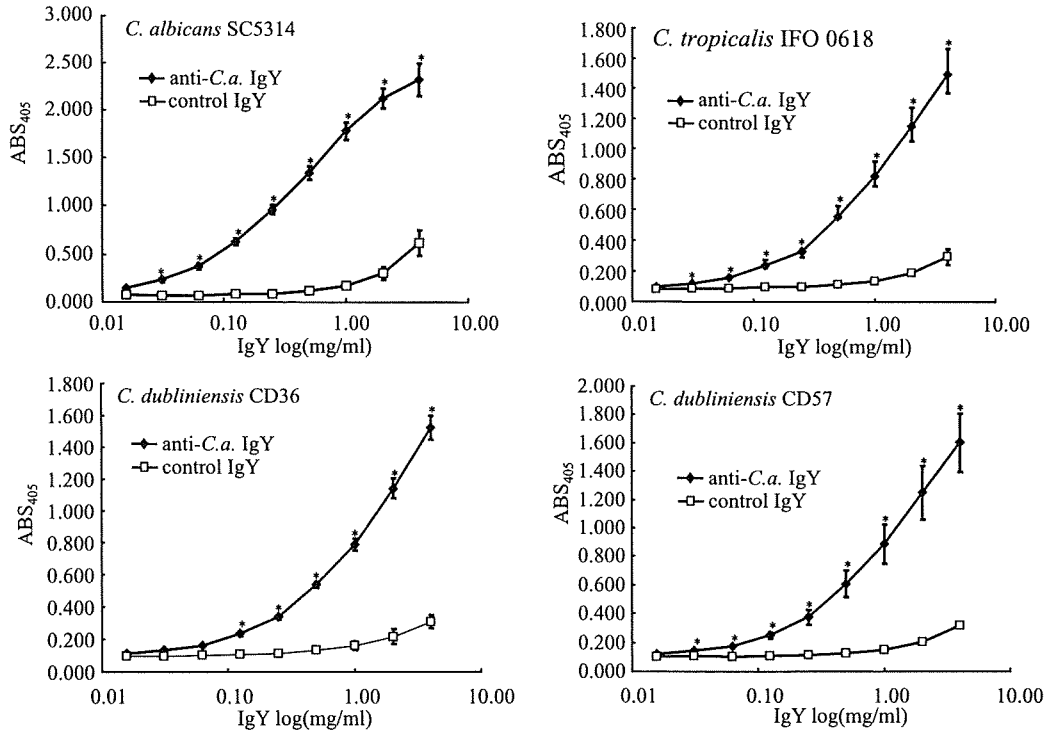

Fig. 1. ELISA antiboby titer of anti-*C. albicans* IgY. Various protein concentration (0.0, 0.063, 0.0125, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/ml) of anti-*C. albicans* IgY or control IgY were applied to 96-well microtiter plates coated with *C. albicans* SC5314, *C. tropicalis* IFO0618, *C. dubliniensis* CD36 and CD57. The titers were determined using a microplate reader at 405 nm. Results are the mean ± standard deviation of three independent experiments each performed using triplicate assays; and compared to control IgY (*$P < 0.01$).

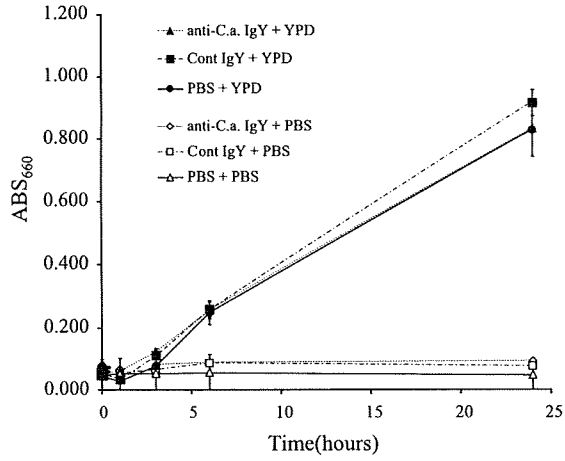

Fig. 2. Effects of anti-*C. albicans* IgY on *C. albicans* growth. Cell suspensions of *C. albicans* SC5314 were mixed with 0 or 2 mg/ml anti-*C. albicans* IgY or 2 mg/ml control IgY; and incubated in YPD or PBS for 24 h. The absorbance at 660 nm was measured at 0, 1, 3, 6 and 24 h after incubation. Results are the mean ± standard deviation of three independent experiments each performed using triplicate assays.

medium containing control IgY or PBS (Fig. 2). *C. albicans* did not grow in PBS containing anti-*C. albicans* IgY, control IgY and PBS. To confirm the reactivity of the anti-*C. albicans* IgY induced by immunization with the yeast form, a reaction assay using a second fluorescence-conjugated antibody was performed and observed by microscopy. The fluorescence activity did not appear in the assay using the control IgY (Fig. 3A). By contrast, significant fluorescence on the yeast form was confirmed using the anti-*C. albicans* IgY (Fig. 3B). Therefore, the reactivity of anti-*C. albicans* IgY to the *C. albicans* yeast form was confirmed. Further, the effect of anti-*C. albicans* IgY on the adherence of *Candida* spp. to monolayers of Ca9-22 epithelial cells was observed (Fig. 4). Anti-*C. albicans* IgY inhibited the adherence of *C. albicans* in a dose-dependent manner (from 0 to 2 mg/ml) whereas 2 mg/ml of control IgY and PBS did not inhibit the adherence. Further, anti-*C. albicans* IgY significantly inhibited the adherence of various *Candida* strains including different serotype strains (A and B) of *C. albicans* in comparison with control IgY (Fig. 5A). Inhibition of adherence was also observed in the other epithelial cell line, HSC-2 (Fig. 5B). To measure inhibition effects of anti-*C. albicans* IgY on biofilm formation of *C. albicans*, various concentrations of anti-*C. albicans* IgY were applied into the biofilm formation. Two milligrams per milliliters of anti-*C. albicans* IgY strongly inhibited the biofilm formation of *C. albicans* SC5314 in comparison with 2 mg/ml of control IgY (Fig. 6). Other concentrations of anti-*C. albicans* IgY did not affect the biofilm formation. Therefore, 2 mg/ml of anti-*C. albicans* IgY is a sufficient amount to inhibit biofilm formation. It is known that serum induces germ tube formation (filamentous form) in *Candida* (33). To detect the reactivity of the anti-*C. albicans* IgY to the germ tube, *C. albicans* was cultivated in medium supplemented with 10% FBS and mixed with the antibody. Yeast and filamentous forms were observed in Fig. 3C. The fluorescence activity of the filamentous form was lower than that of the yeast form of *C. albicans* in the assay using the anti-*C. albicans* IgY (Fig. 3C). To determine whether the IgY anti-*C. albicans* antibody affects the biofilm formation including the germ tube formation of *C. albicans*, we performed further experiments. The biofilm formation assay was performed in a medium conditioned with 10% FBS. Slight inhibitory activity by anti-*C. albicans* IgY was observed for the concentrations 0.25 and

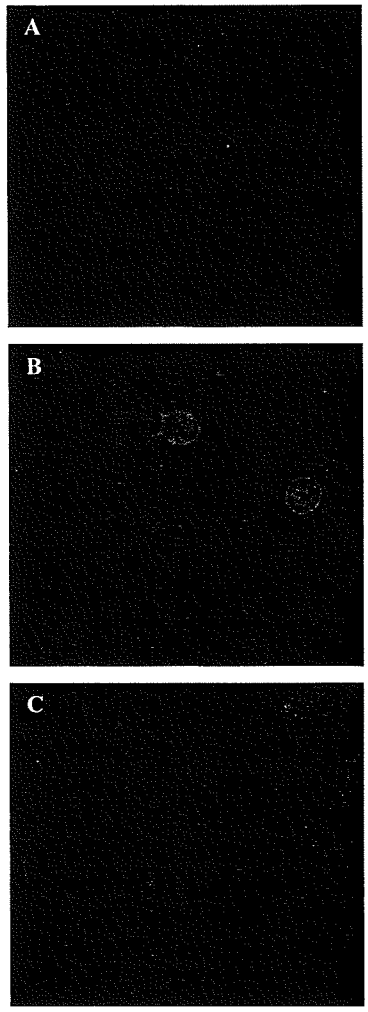

Fig. 3. Photograph of *C. albicans* with FITC-conjugated antibody. *C. albicans* SC5314 was treated with control IgY (A) or anti-*C. albicans* IgY (B). *C. albicans* formed germ tube was treated with anti-*C. albicans* IgY (C). After washing with PBS, the cells were mixed with FITC-conjugated rabbit anti-chicken IgY antibodies. Fluorescence photograph of *C. albicans* treated with antibodies were representative in three independent experiments.

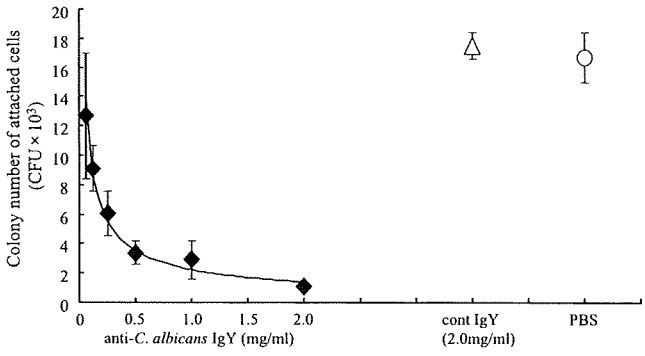

Fig. 4. Effects of anti-*C. albicans* IgY on *C. albicans* adherence. *C. albicans* SC5314 was mixed with 0.0, 0.063, 0.125, 0.25, 0.5, 1.0 and 2.0 mg/ml anti-*C. albicans* IgY, 2.0 mg/ml control IgY or PBS; and applied onto the epithelial cells (Ca9-22). The cell suspension detached using 0.05% trypsin-EDTA were spread on YPD agar plates. After incubation for 24 h, the numbers of colonies on the plates were counted. Results are the mean ± standard deviation of three independent experiments each performed using triplicate assays.

0.5 mg/ml ($P < 0.05$). The 2 mg/ml concentration of anti-*C. albicans* IgY significantly inhibited biofilm formation in the

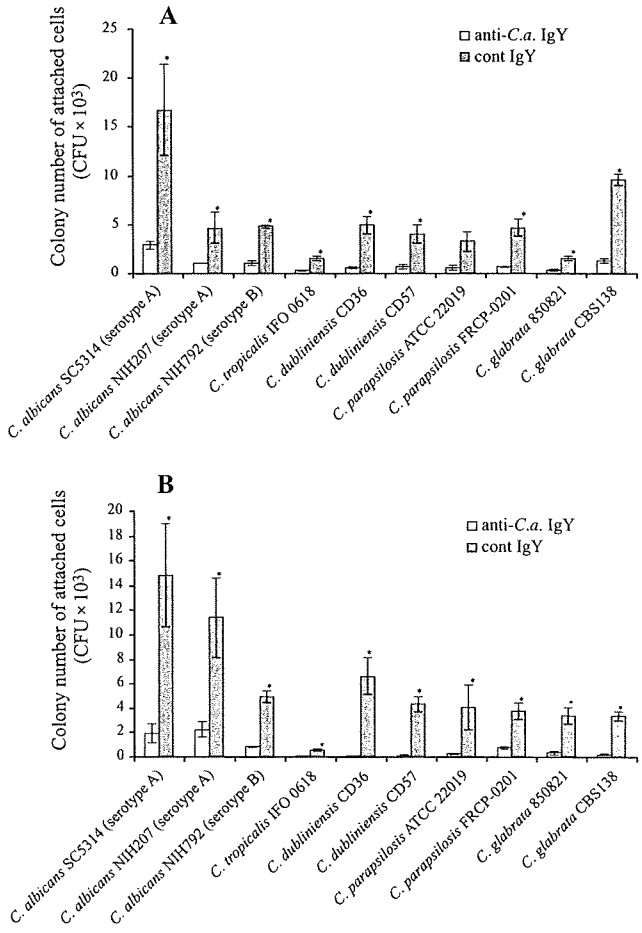

Fig. 5. Effects of anti-*C. albicans* IgY on *Candida* spp. adherence. *Candida* spp. were mixed with 2.0 mg/ml anti-*C. albicans* IgY or 2.0 mg/ml control IgY for 60 min. The treated cells were added onto monolayers of the epithelial cells {(A) Ca9-22 and (B) HSC-2}. The cell suspensions were detached using 0.05% trypsin-EDTA; and were spread on YPD agar plates. After incubation for 24 h, the numbers of colonies on the plates were counted. Results are the mean ± standard deviation of three independent experiments each performed using triplicate assays; and compared to control IgY (*$P < 0.01$).

medium with FBS ($P < 0.01$), but the inhibiting activity was weak in comparison with that by 2 mg/ml of anti-*C. albicans* IgY in the medium without FBS (Fig. 6). PBS did not affect biofilm formation in medium with or without FBS.

DISCUSSION

A number of secretory antibody-mediated mechanisms are operative in the mammary gland including (i) anti-adhesive activity, (ii) opsonization followed by phagocytosis, (iii) toxin neutralization and (iv) antibody-mediated lysis of pathogens (34). This study provided evidence for the anti-adhesive activity of anti-*C. albicans* IgY (IgA-like) against *C. albicans*. We found that anti-*C. albicans* IgY inhibits adherence of *C. albicans* and also other *Candida* spp. to monolayers of oral epithelial cells and confirmed that the IgY antibodies cross-reacted with various *Candida* spp. The IgY induced by immunization with *C. albicans* may react with various antigens including adhesins from *Candida* spp. that adhere to epithelial cells. For example, Hwp1 and Als3 are known for their role in host attachment and are the most well characterized *C. albicans* cell surface proteins (35,36). This is possibly the reason for the inhibition mechanism by anti-*C. albicans*

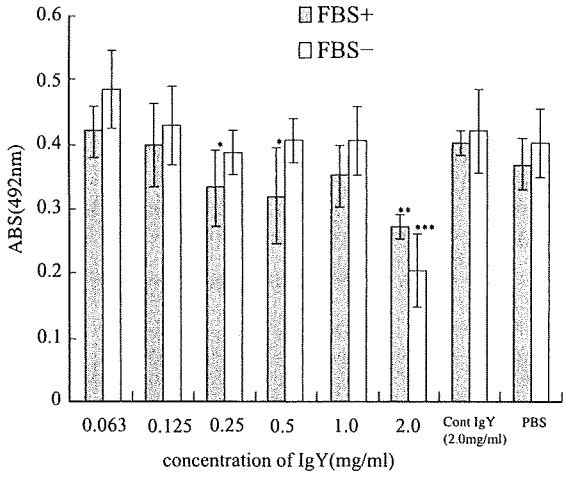

Fig. 6. Biofilm formation of *C. albicans* treated with anti-*C. albicans* IgY. A *C. albicans* SC5314 suspension was added to 0.0, 0.063, 0.125, 0.25, 0.5, 1.0 and 2.0 mg/ml anti-*C. albicans* IgY, 2.0 mg/ml control IgY or PBS to the wells of 96-well microtiter plates. After incubation for 24 h in PBS or YPD with and without 10% FBS, biofilm formation was observed using microphotography. Results are the mean ± standard deviation of three independent experiments each performed using triplicate assays; and compared to control IgY (*$P < 0.05$, **$P < 0.01$ in condition with FBS, ***$P < 0.01$ in condition without FBS).

IgY, where the IgY antibody may cross-react with adhesins Hwp1 and Als3 in the yeast form. The effects may also be associated with the inhibition of biofilm formation in the medium without conditioning serum.

However, the biofilm of *C. albicans* in the medium conditioned with 10% FBS was more resistant to anti-*C. albicans* IgY. A non-dialyzable component of serum induces germ-tube formation using the YPD medium supplemented with 10% serum (28,37). The inhibition of adhesion is usually achieved by blocking the adhesins present on the fungal cell wall (16), but for the inhibition of germination there may be another important mechanism because filamentation plays a key role in the adhesion process in biofilm formation (38). Anti-*C. albicans* IgY induced by immunization with the yeast form is not likely to play an extensive role in the germination of *C. albicans* since it may not include all antibodies to antigens of the filamentous form of *C. albicans* (Fig. 3C). Therefore, the germination might disturb the inhibition by anti-*C. albicans* IgY to biofilm formation in the presence of serum. In contrast to the discrete activity of germination and adhesion to the epithelial cells, anti-*C. albicans* IgY did not exhibit a potent fungicidal effect on *Candida* spp., as it did on *C. albicans*.

Passive immunization therapies against pathogens have been extensively studied (39-41). In the oral cavity, successful passive immunization with IgY against dental caries (e.g., *Streptococcus mutans*) has been reported in a rat model (42,43) and in human subjects (44). Oral passive immunization of anti-*C. albicans* IgY was shown to be effective (27) and significantly reduced the number of *C. albicans* colonies and the scores for tongue lesions. They indicated that this effect may be due to the blocking of the binding of *C. albicans* to the host cells. Here, we demonstrate that anti-*C. albicans* IgY has anti-adherence activity against various *Candida* spp. strains, both when grown in suspension and as a biofilm in the medium without serum. However, these concentrations of IgY did not achieve *Candida* growth inhibition. Chicken egg yolk immunoglobulin is recognized as an antibody source and showed therapeutic values against several microorganisms (19-26). It is possible the anti-*C. albicans* IgY may be used as a preventive immunotherapy against oral and disseminated candidiasis and *Candida* spp. infections. However, the IgY did not completely affect the biofilm formation when *C. albicans* formed germ tubes in the growth medium conditioned with serum. Therefore, treatment with anti-*C. albicans* IgY may be considered a prophylactic immunotherapy or possibly an adjunctive anti-fungal therapy under limited conditions.

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Original Article

Longitudinal Evaluation of Multi-phasic, Odontological and Nutritional Associations in Dentists (LEMONADE Study): Study Design and Profile of Nationwide Cohort Participants at Baseline

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ABSTRACT

Background: To examine the association between oral health and general well-being, we are currently conducting a nationwide cohort study comprising members of the Japan Dental Association (JDA). Herein, we describe the study design and the profile of the participants at baseline.

Methods: From 2001 through 2006, the participants completed a baseline questionnaire that surveyed factors related to lifestyle, general health, and oral health. Morbidity and mortality have been monitored by using information from fraternal insurance programs operated by prefectural dental associations. All respondents provided written, informed consent for participation and the use of their insurance data.

Results: A total of 21 272 JDA members participated in the baseline survey (response rate, 36.2%). Their mean age ± SD was 52.3 ± 12.3 years; 8.0% were women. Among the respondents, 30.2% of men and 10.7% of women were current smokers; 73.5% of men and 44.8% of women were current drinkers. The cohort scored higher on oral health indices than did the general Japanese population: dentists were more likely to brush their teeth ≥3 times/day, to have ≥20 teeth, to have fewer lost teeth, to be free from periodontal diseases, and to have higher General Oral Health Assessment Index scores. There was, however, considerable inter-individual variation in scores on the indices.

Conclusions: More than one-third of JDA members participated in the study. Their oral average health status was better than that of the general population. Nevertheless, it will be possible to compare morbidity and mortality between those with better and worse scores on oral health indices.

Key words: oral health; general well-being; diet; cohort studies; dentists

INTRODUCTION

Oral health is now known to be related to systemic diseases.[1] Tooth loss may result in an unbalanced diet,[2–4] which in turn may lead to chronic diseases such as cardiovascular disease and cancer. Periodontal infections may adversely affect glucose tolerance and accelerate hyperlipidemia[5] and atherosclerosis.[6] These effects may be mediated by endotoxins, such as lipopolysaccharide, and/or inflammatory cytokines, such as tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6.[5,7] Poor oral health has also recently been associated with total mortality.[8,9]

The associations of periodontal disease and tooth loss with the incidence of systemic diseases, particularly cardiovascular disease[8,10] and cancers,[8,11] and with total mortality[8,9] have been examined in case-control and cohort studies, but the results were inconsistent. Cohort studies are superior to case-control studies because they avoid recall and selection biases. A prospective study of the relation between oral health and systemic diseases or premature death in the general population

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would be quite costly, as it would require a considerable number of participants, individual oral examinations by dentists or dental hygienists, and painstaking long-term follow-up for target diseases or death.

We thus attempted to conduct a nationwide prospective study among Japanese dentists to examine the association of oral health with total mortality and systemic diseases, including cardiovascular disease, cancer, and other diseases. Because the participants were dentists, information on oral status could be obtained by means of a questionnaire survey. In addition, information on mortal and morbid events could be collected easily from fraternal insurance organizations operated by the participants' affiliated dental associations. Moreover, we assumed that dentists would be more interested than the general population in the association between oral and general health. Similar cohorts of physicians,[12] nurses,[13] and various healthcare professionals including dentists[13–15] have provided valuable information. In this article, we describe the study design and the profile of cohort participants at baseline, including information on demographic characteristics, lifestyle, general health status, and oral health. Our study was designated the Longitudinal Evaluation of Multi-phasic, Odontological and Nutritional Associations in Dentists (LEMONADE) and the participants are now followed for outcome events.

METHODS

Participants and baseline survey

The participants of the present study were members of 46 prefectural dental associations affiliated with the Japan Dental Association (JDA). The JDA is a unique dental professional organization that principally comprises dental practitioners; it had enrolled 67.2% of all dentists in Japan at the end of 2006. The study participants completed a baseline questionnaire distributed by prefectural dental associations and were registered from February 2001 through July 2006. The questionnaire was used to collect information on demographic factors, including sex, year of birth, age, marital status, and number of members in household; on occupational environment, including length of career as a dentist, employment (independent or employed), number of holidays per month, working hours per week, and number of employees (if any); on physiological status, including height, weight, and usual blood pressure (participants were not instructed to re-measure for the survey); on current history of and medication for chronic diseases including stroke (cerebral infarction, cerebral hemorrhage, and subarachnoid hemorrhage), myocardial infarction, hypertension, diabetes mellitus, hyperlipidemia, and cancer; on family history of cardiovascular diseases, hypertension, diabetes, and cancer; on lifestyle factors including smoking and alcohol drinking habits, sleeping hours, physical activity, consumption of soft drinks, number of breakfasts per week, and dietary intake; on subjective health status including health-related quality of life (QOL), on psychological distress, as measured with the 12-item General Health Questionnaire (GHQ-12)[16]; and on indicators of oral health.

The section of the questionnaire on diet inquired about the intake frequency of 97 common Japanese foods and dishes during the one month preceding the baseline survey. This food frequency questionnaire was designed to estimate daily food group consumption and nutrient intakes, and has been validated.[17,18] The oral health factors included in the questionnaire included oral hygienic routines, such as frequency of brushing, flossing, and scaling; number of teeth lost (excluding third molars); use of dentures; dental examination chart; periodontal status classified according to the criteria of the Community Periodontal Index (CPI)[19,20]; history of periodontal diseases with pockets ≥4 mm; and oral health-related QOL, as determined by using the Japanese version of the General Oral Health Assessment Index (GOHAI).

The dental examination chart was recorded in the participant's office. Participants were asked to assess their periodontal status and to classify it according to the CPI criteria, which were described in the questionnaire. Periodontal status was assessed for 6 teeth in the dentition, as specified in the WHO protocol for CPI measurement.[19] Each segment was then designated as healthy (score 0), bleeding but without dental calculus (score 1), with calculus but without pockets (score 2), with pockets 4.0 to 5.9 mm in depth (score 3), or with pockets ≥6.0 mm in depth (score 4), according to the highest score for an index teeth. The highest score among 6 segments was regarded as the CPI score of the participant. In 41 of the 46 prefectural dental associations, the profession of the individual who assessed the periodontal status (dental hygienist, another dentist, the participant, or other) was also recorded on the questionnaire.

The Japanese version of the GOHAI questionnaire has been adapted to and validated in the Japanese population,[21] and the national norm for Japanese is obtainable through iHOPE International (Tokyo and Kyoto). GOHAI is a 12-item index summarized in 3 dimensions: (1) physical function, which includes eating, speech, and swallowing, (2) psychosocial function, which includes anxiety or concerns about oral health, self-image, and self-consciousness about oral health, and (3) avoidance of social contacts because of oral problems, and pain or discomfort. Each item was scored from 1 to 5 and the scores were then summed for all items. The total score, therefore, ranges from a minimum of 12 to a maximum of 60. A higher total score indicates better oral health-related QOL.

Validation study of self-reported periodontal status

To validate the assessment of periodontal status reported on the self-administered questionnaire, we compared the CPI score reported on the questionnaire with the CPI score measured in an oral examination by one of the authors (TN).