INTRODUCTION

The understanding of periodontal condition including natural history is growing rapidly (MIyazaki *et al.* 1989, 1995). Periodontal destruction is a frequent experience among elderly people (Slade *et al.*, 1995; Brown *et al.*, 1996). It is contributes to as many as 40 percent of extraction (Jonson, 1993). Nevertheless, elderly people who show minimun periodontal tissue destruction certainly exist (Papapanou and Lindhe, 1992).

It is as yet impossible to effect any elevation in host resistance. However, If a role for genetic polymorphism in the pathogenesis of complex disease such as periodontal disease can be established, the information on the multiple genetic determinants will be useful in understanding the aberrant function of genes and how this affects homeostasis.

Neutrophils play an important role in the control of periodontitis, and increased disese susceptibility is observed in patients with defective neutrophil production and/or fuction (Hart *et al.*, 1994; Van Dyke *et al.*, 1994; Kinane, 1999). Neutrophils constitute approximately 90 % of immunocompetent cells in gingival crevicular fluid. In addition, Neutrophils are the first leukocytes that infiltrate inflammatory sites, and are also found in healthy gingival crevice (sugita *et al.*, 1993). Therefore, genetic polymorphism that affect neutrophil effector fuction might be relevant for disease resistance.

FcγRIIIb is a neutrophil-specific receptor and bears the functional NA1-NA2 polymorphism which determines IgG1- and IgG3-mediated neutrophil effector function. The NA1-NA2 polymorphism caused by 4 amino acid substitutions within the first extracellular Ig-like domain. In our previous reports, we demonstrated a role of FcγRIIIb allele as a risk factor for periodontal disease (Kobayashi *et al.*, 1997, 2000a, 2000b, 2001; Sugita *et al.*, 1999, 2001; Yoshihara *et al.*, 2001).

In our previous study for elderly population, we found significant associations between additional attachment loss during two years and smoking, and attachment level of 6 mm or more at baseline (Ogawa *et al.*, 2002 in press). In the same group, we found that the subjects who had over than 20 remaining teeth were relevant for periodontal disease resistance (Hirotomi *et al.*, 2002 in press). In addition, we found that FcγRIIIb-NA1 allotype was over-represented in the periodontitis-resistant group, compared with the priodontitis-susceptible group according to cross-sectional study (Sugita *et al.*, 2001).

The purpose of this study is to determine how FcγRIIIb-NA1-NA2 polymorphism and smoking are associated with periodontal disease progression among healthy elderly people longuitudinally.

MATERIAL & METHODS

Subjects and Clinical Assessment

In 1998, in an oral health survey of the elderly population conducted by the Ministry of Health and Welfare of Japan, we sent questionnaires to all 6,629 residents ageds 70 or 80 years old in Niigata City (Japan) regarding their medical and dental health conditions.

Among these, 599 persons 70 years of age agreed to undergo the medical and dental examinations (screened population), with signed informed consent to the protocol, which was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Niigata University. Four dentists performed clinical evaluations on the following items: ① unmber of teeth present, ② probing pocket depth (PPD), and ③ probing attachment level (PAL). PPD and PAL were assessed by means of a Williams probe at six sites per tooth and recorded to the nearest millimeter.

Among the screened population, 309 subjects with neither diabetes mellitus nor blood sugar ≥140mg/dL were randomly selected. In addition, the only persons with more than 20 teeth present included in the study. The FcγRIIIb-NA1/NA2 genotype was determined.

Among the subjects included in the study, NA1 group was defined as having NA1NA1 genotype (NA1 group), whereas the NA2 group was defined as having NA1NA2 or NA2NA2 genotype. Number of the subjects in the study were 164 (NA1 group: 54, NA2 group: 110). Follow-up clinical surveys were carried out by PAL after three years.

Prior to data collection, the four examiners were calibrated with each examiner paired with all other examiners on 17 volunteer patients in the Faculty Hospital of Dentistry, Niigata University. The percentage of agreement ranged from 85.5 to 100% for PPD and from 70.0 to 100% for PAL. The kappa ranged from 0.77 to 1.00 for PPD and from 0.62 to 1.00 for PAL.

Determination of FcγRIIIb-NA1/NA2 Genotype and smoking habit

Genomic DNA was isolated from peripheral blood (Easy-DNA kit; Invitrogen, SanDiego, CA, USA) and genotyped for FcγRIIIb-NA1-NA2 by allele-specific polymerase chan-reaction, as previously described (de Haas *et al.*, 1995; Kobayashi *et al.*, 1997). Furthermore, an personal interview was performed to obtain the bulk of information regarding smoking.

Statistical Analysis

We used the Mann-Whitney U test to compare the clinical parameters between the NA1 and NA2 groups at baseline. In addition, the proportion of subjects experiencing ≥

3mm or ≥ 5mm additional attachment loss at either sites during three years were calculated.

For evaluating how FcγRIIIb-NA1-NA2 polymorphism and smoking are associated with periodontal disease progression, progressive person rate with more than eleven ≥ 3mm additional attachment loss sites or more than one ≥ 5mm additional attachment loss site during three years between smoker and no smoker in the NA1 or NA2 groups were calculated.

The relative risk for periodontal progression with more than eleven additional attachment loss sites of ≥3mm during three years between smoker and no smoker in the NA1 or NA2 groups was calculated. In addition, the relative risk for periodontal progression with more than one additional attachment loss site of ≥5mm during three years between smoker and no smoker in the NA1 or NA2 groups was calculated.

RESULTS

As shown in Table 1, there were no significant difference in the clinical parameters between the NA1 and NA2 groups at baseline. After dividing the subjects into smoker and no smoker, we evaluated the progressive rate who had ≥3mm AAL during three years (Fig 2). The progressive rate in smoker was 0% for NA1 group, 35.0% for NA2 group (Fisher's exact probability=0.087). Table 2 shows the relative risk of smoker or no smoker with NA1 or NA2 genotypes who had over than 11 ≥3mm AAL. The relative risk between smoker and no smoker in the NA2 group was 2.06 (Fisher's exact probability=0.009).

The progressive rate in smoker who had ≥5mm AAL during three years was 0% for NA1 group, 35.0% for NA2 group (Fig 2, Fisher's exact probability=0.087). The table 3 shows the relative risk of smoker or no smoker with NA1 or NA2 genotypes who had ≥5mm AAL during three years. The relative risk for periodontal progression with more than one ≥5mm AAL during three years between smoker and no smoker in the NA2 group was 3.93 (Fisher's exact probability=0.006).

DISCUSSION

FcγRIIIb is the predominant FcγR on neutrophis, which are present of high numbers in gingival crevicular fluid and subjacent to the apical part of the pocket epithelium (Sugita et al., 1993, Yuan et al., 1999). In our study, the subjects with NA2 genotype had higher progressive rate than the subjects with NA1 genotype especially in smoker. The progressive rate for more than eleven ≥3mm additional attachment loss sites (AAL) in smoker was 0% for NA1 group, 35.0% for NA2 group. In addition, the progressive rate in smoker who had ≥5mm AAL during three years was 0% for NA1 group, 35.0% for NA2 group. These findings suggested the FcγRIIIb-NA1-NA2 polymorphism to contribute to periodontal progression. FcγRIIIb, of which function and roles in periodontitis have demonstrated previously, are expected to be reliable as a candidate genetic facor for progression of periodontitis in elderly Japanese.

Furthermore, smokers had a higher progressive rate especially in the NA2 group. The relative risk was 2.06 for ≥ 3mm AAL, 3.93 for ≥ 5mm AAL. The previous reports indicating a strong association between smoking and periodontal disease progression. Smoking was found to be the most important explanatory variable for periodontal disease progression and modification of this factor is important in the treatment and prevention of periodontal disease. The strong association between smoking and subsequent attachment loss could be explained by a number of biologic phenomena. Nicotine, the chief noxious substance found in cigarettes, and its byproducts have a vasoconstrictive effect, not only on peripheral circulation, but also on coronay, placental, and gingival blood vessels (GonzClez *et al.*, 1996). In addition, according to The Research, Science and Therapy Committee of the American Academy of Periodontology 1996, nicotine may reduce the functional activity of leukocytes and macrophages both in saliva as well as crevicular fluid, and it decreases chemotaxis and phagocytosis of blood and tissue polymorphonuclear leukocytes. Accroding to our study, smoking might reinforce the function of FcγRIIIb-NA2 allele due to reduce the functional activity of leukocytes and macrophages. It should be noted that the reason why smoking had influenced the function of FcγRIIIb-NA2 polymorphism stronger than the function of FcγRIIIb-NA1 polymorphism.

Other polymorphisms than FcγRIIIb-NA1-NA2 have been recently shown to be risk factors for periodontitis as well. IL-1 genotype (Kornman *et al.*, 1997; Cullinan *et al.*, 2001), FcγRIIa 158V-F polymorphism (Sugita *et al.*, 1999), fMLP polymorphisms (Gwinn *et al.*, 1999), vitamin D receptor (Hennig *et al.*, 1999) have been shown to be associated with periodontitis. Not only each polymorphism, but also combining with some polymorphisims might significantly affect the incidence (Yoshihara *et al.*, 2001). Another survey with a larger number of subjects may be necessary to evaluate the mechanism how genes influence periodontal progression.

In summary, our results support the FcγRIIIb-NA1-NA2 polymorphism may be associated with periodontal progression in elderly especially in smoker. Further studies should be undertaken to confirm these observations in different ethnic backgrounds.

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Table 1 Biographical and periodontal characteristics of each subject group at baseline.

Parameters	Screened population (n=599)	Subjecyts included in the study (n=309)	Subjects for analysis (n=164)		p value (NA1 vs. NA2)
			NA1NA1	NA1NA2 or NA2NA2	
Male/Female	306/293	157/152	30/24	61/49	0.990
Smokers (%)	18.7	19.7	13.0	18.2	0.990
Subjects under medical treatment for diabetes (%)	5	None	None	None	-
Subjects with blood sugar >=140 mg/dl(%)	7	None	None	None	-
Edentulous subjects (%)	7.5	12.9	None	None	-
Number of teeth present (mean±SD)	17.4±0.4	18.3±0.6	25.96±2.80	25.08±2.91	0.067
Subjects with >=20 teeth present (%)	49.6	68.9	All	All	-
Probing pocket depth, PPD mm (mean±SD)	2.03±0.03	2.00±0.04	1.88±0.47	1.91±0.51	0.680
Probing attachment level, PAL mm (mean±SD)	3.10±0.05	3.04±0.08	2.58±0.85	2.72±0.78	0.296
Number of sites (mean±SD)					
PPD >=4mm	9.63±12.62	9.65±13.33	10.63±12.94	9.86±14.20	0.739
PPD >=6mm	1.29±3.01	1.09±3.08	0.83±2.05	1.09±3.50	0.618
PAL >=4mm	28.29±25.23	30.92±31.13	30.74±33.71	33.46±30.62	0.606
PAL >=6mm	5.74±9.39	5.28±10.19	5.41±11.66	4.65±8.28	0.635

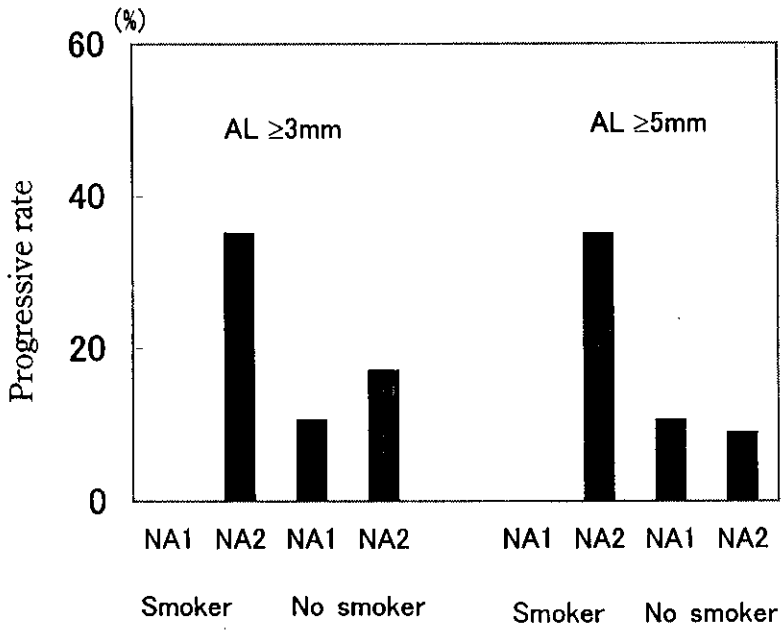

Fig 1. Percentage of persons who have more than eleven ≥3mm additional attachment loss sites, and who have more than one ≥ 5mm additional attachment loss between NA1 and NA2 groups in smoker or no smoker. There were higer trends in progressive rate between NA1 group and NA2 group in smoker for both AL ≥3mm and AL ≥5mm ($p=0.087$ by Fisher's exact probability).

Table 2 Relative risk for eleven ≥3mm additional attachment loss sites between smoker and no smoker.

		0*	1-10*	11-*	Relative risk (Fisher's exact probability)
NA1NA1	Smoker	0**	7	0	- (0.302)
	No smoker	8	31	8	
NA1NA2 or NA2NA2	Smoker	3	10	7	2.06 (0.009)
	No smoker	21	60	9	

- (0.087)

1.60 (0.180)

* Number of sites which have ≥3mm additional attachment loss during three years.

** Number of subjects.

Table 3 Relative risk for one ≥5mm additional attachment loss sites between smoker and no smoker.

		0*	1-*	Relative risk (Fisher's exact probability)
NA1NA1	Smoker	7**	0	- (0.485)
	No smoker	42	5	
NA1NA2 or NA2NA2	Smoker	13	7	3.93 (0.006)
	No smoker	82	8	

- (0.087)

0.84 (0.479)

* Number of sites which have ≥5mm additional attachment loss during three years.

** Number of subjects.

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E．研究目的：

高齢者は，さまざまな慢性疾患や機能低下により，日常の生活に支障を来すことがよくある。しかし，う蝕や歯周疾患などの歯科疾患と栄養状態や慢性感染症，運動機能といった全身的な健康状態との関連についてはまだよくわかっていない。本調査では，全身的な健康状態の変化とう蝕や歯周疾患との関連について検討することを目的としている。特に今回は，全身の健康状態を表す指標として血清アルブミン値を採用した。血清ｱﾙﾌﾞﾐﾝ値に関しては，近年の疫学的調査結果から，加齢とともに低下傾向を示すことが報告されている。高齢者では総死亡率に関して独立した危険因子であることが明らかになっている。慢性感染症や低栄養状態との関連を報告した調査も多くある。この点を踏まえ，高齢者における血清ｱﾙﾌﾞﾐﾝ値とう蝕や歯周疾患との関連を評価したいと思う。

F．研究方法：

新潟市内在住の70歳と80歳の高齢者を対象とした。事前に，市内在住の70歳，80歳全員，6,629 名に郵送によるアンケート調査をおこない，調査参加希望者を中心に男女の比率が同じになるように対象者を選出した。選出された対象者は70歳600名，80歳163名である。

本調査では，診査項目として，12 の指標を設定した。まず，血清アルブミン値については，調査対象者全員に対し採血を行い，ただちに血清分離をした後，BCG 法を用いて測定をおこなった。その他，身長と体重から算定するＢＭＩ指標，免疫機能ではIg GおよびIg A を指標として採用した。また，食物摂取状況を示す指標として，１日あたりの脂質，糖質，蛋白質の摂取量を算定した。その際，簡易食事評価法である食物摂取状況調査票およびフードモデルを用い，栄養士による聞き取り調査で行った。

次に，口腔の健康状態を示す指標として，根面う蝕については，ＷＨＯの基準に従い，未処置歯，喪失歯，処置歯数を検出した。

分析にあたっては，まず，70 歳と 80 歳別に血清ｱﾙﾌﾞﾐﾝ値と各種測定値との関連を評価した。次に，血清ｱﾙﾌﾞﾐﾝ値を従属変数に，年齢，性別，未処置歯数，喪失歯数，処置歯数，IgG，IgA，BMI，脂質摂取量，蛋白摂取量，糖質摂取量を独立変数とした

重回帰分析を行った。

G．研究結果・考察：

血清アルブミン値と各指標との関連をみると，年齢および性別でコントロールした分散分析で統計学的に有意であった指標は，未処置歯数，Ig G，BMI，であった。未処置歯数を０本，１～２本，３本以上の３つのカテゴリーに分類した場合に，未処置歯数の増加とともに血清アルブミン値の低下する傾向が確認でき，未処置歯数が０本と３本以上での血清アルブミン値の差は，70 歳では 75.56mg/dl，80 歳では 202.97mg/dl であった。

我が国の一地域で行われた，70 歳の高齢者を 10 年間追跡した疫学調査では，血清ｱﾙﾌﾞﾐﾝ値の 100mg/dl オーダの差がその後の余命に関連していた。このことからみても本調査結果の差は決して小さくはないと考えられる。

H．結論：

8020 データバンク調査に伴い新潟市に住む 70 歳，80 歳の中で，研究への協力に合意した 763 人が採血・歯周診査を含む一連の検査を受けた。歯科疾患の中でも特に未処置歯数が血清ｱﾙﾌﾞﾐﾝ値と有意に関係していることが確認できた。このことは，全身的な健康状態の変化がう蝕発生のリスク要因となる可能性を示唆している。

I．研究発表論文：

投稿原稿

Association between serum albumin and root dental caries in elderly people living in the community

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A running heading: serum albumin and root dental caries

Abstract

Background: the root caries incidence was reported as a risk predictor for mortality from recent study. This may indicate a link between general health and root caries. However, the relationship between a dental disease such as root caries with the general health condition, including nutrient intake or anthropometry, was unknown.

Objectives: to evaluate the relationship between the so-called general condition and the root caries.

Design: initially, questionnaires were sent to all 6,629 inhabitants aged 70 and 80 years old according to a registry of residents in Niigata city in Japan, and they were informed of the purpose of this survey and ask whether they wanted to participate. According to the replies, 763 individuals were selected randomly in order to have approximately the same numbers of gender of each age.

Body Mass Index, serum albumin, Ig G and Ig A for Body composition and blood measurements, total daily nutrient intakes of sugar, fat and protein from food for food intake, and root dental caries were measured.

Main outcome measures: the relationship between root caries and serum albumin concentration was evaluated by multiple linear regression analysis.

Results: the differences in the serum albumin concentration between subjects with the number of untreated root caries (DT)=0 and DT $\geqq$ 3 were 75.56mg/dl in 70 yrs and 202.97mg/dl in 80 yrs. For multiple linear regression, four variables (number of untreated root caries, age, Body Mass Index and concentration of IgG) were selected for the independent variables of the final model. The number of untreated root caries was significantly associated with the concentration of serum albumin ($p<0.05$).

Conclusions: the number of untreated teeth was a significant factor associated with serum albumin concentration. The difference in serum albumin levels have a meaningful influence to general status accoring to previous reports. The findings of the present study indicated that a relationship between root caries and general health status of these elderly subjects is highly possible.

Key words: Serum albumin, Root dental caries, Elderly people

Introduction

Disabilities in daily living occur frequently in elderly people because of inflammatory states or disorders. Regarding oral health, elderly people have few remaining teeth. The dental caries including root caries was significantly associated with incidence of tooth loss (Hand *et al.*, 1991; Locker *et al.*, 1996).

Furthermore, the root caries incidence was reported as a risk predictor for mortality from recent study (Mauriello *et al.*, 1999). This may indicate a link between general health and root caries. However, the relationship between a dental disease such as root caries with the general health condition, including nutrient intake or anthropometry, was unknown.

The purpose of this study is to evaluate the relationship between the so-called general condition and the root caries. Especially, we adopted the serum albumin concentration as a criteria which shows us the general condition. The serum albumin may be an index of the severity of an underlying disease. In addition, a strong association between albumin level and mortality has been reported (Phillips *et al.*, 1989; Darnes and Ducimetiere, 1990). Many conditions, such as malnutrition, inflammatory states, liver diseases, and renal diseases, reduce serum albumin level (Francois *et al.*, 1992). We evaluated the relationship between the serum albumin and root caries.

Methods

Initially, questionnaires were sent to all 6,629 inhabitants aged 70 and 80 years old according to a registry of residents in Niigata city in Japan, and they were informed of the purpose of this survey and ask whether they wanted to participate. According to the replies, 763 individuals were selected randomly in order to have approximately the same numbers of gender of each age. Written informed consent was obtained from all subjects. The protocol of this study was approved by the Ethics Committee of Niigata University School of Dentistry.

Root caries

Four trained and experienced dentists assessed the oral health conditions of the subjects. Dental clinical examinations was done using dental mirrors and WHO ball-pointed periodontal probes under artificial light, without bite-wing radiographs. Root caries was diagnosed using WHO criteria (World Health Organization, 1997). First, it was detected whether a given surfaces was exposed or not. An exposed root surface was defined as at least 1 mm of visible root surface between the gingival crest and the cement-enamel junction or the restoration margin. All exposed root surfaces were examined and recorded by each surface. Root decay was defined when a lesion was detected on an exposed root surface and felt soft or leathery to probing. For a single decay affecting both the crown and the root, the likely site of origin of the lesion was recorded as decayed. In the case of filling involving both the crown and the root, the most likely site of the primary carious lesion was recorded as filled. The examiners were calibrated by eighteen volunteer patients in the University Hospital before and during the survey. Interexaminer reliability for surfaces was assessed for four examiners. Kappa values between each pair of examiners were 0.56-0.75 for root caries.

Body composition and blood measurements

Anthropometric evaluation included measurements of weight and height to calculate the Body Mass Index. In addition, determinations of serum albumin, Ig G and Ig A were also made. The serum albumin concentration was measured by a BCG method (Doumas *et al.*, 1971) using a Gemsack First Auto-analyzer.

Food intake

Participants were asked to record their own dietary intake on one day by dieticians. The interviewer provided each person with written directions and food

models for recording food intake and reviewed the completed records with each subject. Total daily nutrient intakes of sugar, fat and protein from food were computed for those subjects.

Statistical analysis

For descriptive data (serum albumin and anthropometric measurements, food intake, biochemical measurements, root caries) , statistical differences between age or sex were evaluated by analysis of variance. In addition, the relationship between serum albumin concentration and root caries, missig teeth, Body Mass Index, nutrient intakes, biochemical values were evaluated by multiple liner regression analysis adjusted for age and sex. For evaluation of relationship between root caries and serum albumin concentration, multiple linear regression analysis was performed. As a dependent variable, serum albumin concentration was used. As independent variables, we selected the independent variables, which had p-values less than 0.05 according to the multiple linear regression analysis adjusted by sex and age for each variable.