Yogurt product intake and reduction of tooth loss risk in a Japanese community

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Abstract

Aim: To evaluate the longitudinal association between yogurt product intake and oral health in a population-based study.

Materials and Methods: This study included 1967 Japanese residents aged 40–79 years who underwent dental examinations in 2012. Among them, 1469 participants were followed up in 2017 for the incidence of tooth loss, which was defined as two or more teeth lost over 5 years. The intake of yogurt products, defined as yogurt and lactic acid beverages, was estimated using a semi-quantitative food frequency questionnaire. The composition of the salivary microbiota was evaluated.

Results: The Poisson regression model showed that a higher intake of yogurt products was negatively associated with the incidence of tooth loss (p for trend = .020), adjusted for potential confounding factors. Mediation analysis confirmed that periodontal condition partly mediated the effect of yogurt product intake on tooth loss, while dental caries experience did not. Additionally, we confirmed the association of a high intake of yogurt products with a low percentage of the salivary microbiota pattern, which was associated with poor oral health.

Conclusion: These findings suggest that the intake of yogurt products is associated with a lower risk of tooth loss resulting from periodontal disease, probably via modulation of the oral microbiome composition.

KEYWORDS
microbiota, oral health, periodontitis, yogurt
Clinical relevance

Scientific rationale for study: Consumption of various types of dairy products has been reported to be associated with oral health conditions. The longitudinal association between the intake of yogurt products and oral health has not been well characterized.

Principal findings: A higher intake of yogurt products was found to be associated with a lower risk of tooth loss through periodontal condition. This association might be explained, at least in part, by the probable modulation of the oral microbiome composition.

Practical implications: A higher intake of yogurt products may be beneficial in preventing tooth loss by protecting against periodontal disease.

1 | INTRODUCTION

Tooth loss represents the oral health experience of a lifetime and results from a complex interaction between several causal factors (Kassebaum et al., 2014). Clinical oral conditions, such as dental caries and periodontitis, are directly linked to tooth loss, whereas other factors such as health behaviours and general health problems are indirectly associated (Aida, 2021). An unhealthy diet and lower intake of various nutrients have also been suggested as indirect factors associated with tooth loss (Al-Zahrani et al., 2005; Martinon et al., 2021).

The association between a high intake of fermentable carbohydrates and dental caries is well established (Chapple et al., 2017). The association between vitamin C deficiency and vitamin D or lower dairy product intake and periodontitis is being increasingly recognized (Chapple et al., 2017; Martinon et al., 2021). Dairy products have been proposed to have a beneficial effect on periodontitis through various nutrients, including calcium and other components (Al-Zahrani, 2006). Fermented dairy products are characterized by high concentrations of lactic acid bacteria (Kok & Hutkins, 2018). These live bacteria act as probiotics with beneficial effects on oral health (Nadelman et al., 2018). However, the epidemiological evidence linking the intake of yogurt products and periodontitis is less consistent. Two cross-sectional studies reported that a low intake of yogurt products is associated with periodontitis (Shimazaki et al., 2008; Adegboye et al., 2012).

To date, there have been no longitudinal population-based studies on the intake of yogurt products and oral health conditions, particularly tooth loss. Additionally, epidemiological evidence has not been sufficient to confirm the probiotic effect of yogurt products on the oral microbiota. Nutritional advice for oral health promotion and disease prevention should be based on food or food patterns rather than on single nutrient. Therefore, this study aimed to investigate whether the absolute intake of yogurt products was longitudinally associated with tooth loss, considering oral microbiota in a general Japanese community.

2 | MATERIALS AND METHODS

2.1 | Study population

This study was conducted as a part of the Hisayama Study, a population-based prospective study of cardiovascular disease in the town of Hisayama, a suburb of the Fukuoka metropolitan area in southern Japan. The age, occupational distribution, nutrient intake of the study cohort were similar to those of the general Japanese population according to the national census and nutrition survey (Tomonou et al., 2007; Hata et al., 2013). Medical and dental examinations were conducted at the health centre in Hisayama in 2012. The inclusion criteria were as follows: aged 40–79 years; participation in the nutritional survey of 2012; and the ability to participate in the study (i.e., ability to walk independently, give consent, and answer questions). The exclusion criteria included participants with 10 or fewer teeth and missing data. To analyse the data without the effect of the prosthetic procedure, we excluded 154 participants with 10 or fewer teeth. Almost all of them (n = 146) wore removable dentures. The abutment teeth with clasps are likely to be extracted, as denture rotation by harmful lateral force may result in the resorption of the alveolar bone supporting the abutment teeth (Tada et al., 2015). Additionally, 10 or fewer teeth present may make it challenging to assess current periodontal health properly, and fewer teeth increase the variance of the clinical attachment level (CAL) (the mean CAL in individuals with only a few teeth depends greatly on the number of teeth).

The study protocol was approved by the Kyushu University Institutional Review Board for Clinical Research (Approval No. 28-31). Informed written consent was obtained from all participants.

2.2 | Oral examination

Evaluation of oral health conditions included the number of teeth present, periodontal condition, and dental caries experience. The third molars were excluded from these evaluations. The total number of decayed and filled teeth (DFT) was used to measure dental caries. Examination of the periodontium followed the National Health and Nutrition Examination Survey III method, which included assessment of probing pocket depth (PPD) and CAL of all the teeth (except the third molars) at two sites (mesio-buccal and mid-buccal) (Page &
Eke, 2007). The percentage of teeth that bled upon probing (%BOP) was also assessed. The mean values for PPD and CAL were calculated as the sum of the maximum PPD and CAL per tooth divided by the number of teeth present in each individual. CAL is an estimate of accumulated periodontal tissue destruction, while PD reflects current inflammation in periodontal tissue. Because the association between yogurt product intake and tooth loss is unlikely to be linked with inflammation, CAL was mainly used during the analysis. Eleven trained dentists assessed the periodontal condition (Furuta et al., 2021). The intra-class correlation, used as a measure of inter-examiner reproducibility, was 0.749 for CAL.

### 2.3 Dietary assessment

The dietary survey was conducted using a semi-quantitative food frequency questionnaire. Food items were selected as those commonly consumed in Japan, primarily from the food list used in the National Nutrition Survey of Japan. The validity of this questionnaire has been reported previously (Shirotta & Yoshizumi, 1990). Each participant completed the questionnaire before the examination, and trained dentists checked it during the examination. The yogurt products consisted of yogurt and lactic acid beverages. The intake of dairy products was calculated based on the reported consumption frequency and portion size according to the semi-quantitative food frequency methodology. Calcium intake from dairy products was estimated based on the Standard Tables of Food Composition in Japan, Fourth Revision (Resources Council of Science and Technology Agency, 1982).

### 2.4 Covariates

Information about participants’ toothbrushing frequency, regular dental visits, smoking habits, and occupational status was obtained using a self-administered questionnaire. The frequency of toothbrushing was categorized as once per day or less, and twice per day or more. Participants were categorized as those who did or did not regularly visit the dentist for oral care at least once a year. Smoking status was divided into current smoker, former smoker, or never smoker. Occupational status was classified into three categories: “clerical support workers”, “homemakers, unemployed or retired”, and “other jobs”. Body mass index (BMI) and diabetes were evaluated using clinical and biochemical assessments, respectively. Blood samples were collected after overnight fasting. All participants in this study, except for those with severe diabetes or those undergoing insulin treatment, underwent a 75-g oral glucose tolerance test. Plasma glucose concentrations were determined using the hexokinase method. Diabetes was defined as either undergoing treatment for diabetes with medication and/or insulin injections, a fasting plasma glucose ≥126 mg/dl, 2-h post-prandial or random plasma glucose ≥200 mg/dl, or both (Alberti & Zimmet, 1998).

### 2.5 Salivary microbiota pattern

The saliva sample stimulated by chewing gum for 2 min was collected at the baseline examination. DNA extraction was performed using a bead-beating approach as previously described (Takeshita et al., 2016; Kageyama et al., 2019). The bacterial composition of each sample was determined based on the V1–V2 regions of 16S rRNA gene sequencing analysis using an Ion PGM system (Thermo Fisher Scientific, Waltham, MA). Details of the procedure are described in Supplementary Methods. The bacterial composition in the saliva of all participants who underwent dental examinations and whose saliva samples were collected in 2012 (n = 1940) was classified into community types using an enterotype approach based on Jensen–Shannon divergence and partitioning around medoids clustering as described previously (Arunugam et al., 2011). Consequently, the salivary microbiome was divided into two community types in this study: type I (n = 835) and type II (n = 1105).

### 2.6 Statistical analysis

Tooth loss was evaluated as the difference between the number of teeth present at baseline (in the 2012 survey) and that in 2017. The outcome in this study was the incidence of tooth loss defined by two or more teeth lost (highest quintile of the number of teeth lost) over 5 years. The amount of yogurt product intake was not energy-adjusted, as it was not correlated with the total energy in this study (Spearman correlation coefficient r = 0.01). The participants were grouped into quartiles based on the amount of yogurt product intake per day. The quartiles of yogurt product intake were 0, 0.1–28.6, 28.7–79.9, and ≥80 g/day. The trends in the mean values of covariates for yogurt product intake levels were tested using linear regression and frequencies using logistic regression analysis.

A multivariable Poisson regression model with robust standard errors was used to assess the association between yogurt product intake and the incidence of tooth loss, as defined above. Incidence rate ratios (IRRs) and 95% confidence intervals (CIs) were calculated using the Poisson regression model. The covariates were age, sex, toothbrushing frequency, regular dental visit, smoking, BMI, diabetes, job, number of teeth present and DFT, and mean CAL at baseline. Mediation analysis tests whether the association between an exposure (yogurt product intake) and an outcome (tooth loss) is mediated through a mediator (number of DFT or mean CAL). Analysis of the salivary microbiome community type and path analysis are described in Supplementary Methods. Using the propensity score-matching method to eliminate the effects of confounders, tooth loss was compared between the participants with no intake and those with a high intake of yogurt products, who were matched for toothbrushing frequency, regular dental visit, smoking, and diabetes.

SAS version 9.4 (SAS Institute) was used to perform statistical analyses, and p < .05 was considered statistically significant in all analyses. We followed the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.
RESULTS

There were 2318 participants who fulfilled the inclusion criteria. After excluding 154 participants with 10 or fewer teeth and 197 participants with missing data, 1967 participants completed the baseline examination. Among them, 1469 participants (651 men and 818 women) underwent dental examinations in 2017 (follow-up rate = 74.7%). A flow-chart of the study population is shown in Figure S1.

The participants in the highest quartile of yogurt product intake were older, mostly women, with >1 toothbrushing frequency, with regular dental visits, with a lower percentage of current smokers, more number of DFT, and lower mean CAL, mean PPD, and %BOP, compared to those in the lowest quartile (Table 1).

The cumulative incidence of participants with two or more teeth lost over 5 years was 17.7%. The Poisson regression model showed that the highest quartile of yogurt product intake was negatively associated with tooth loss (IRR 0.73, 95% CI 0.53–0.99 in Model 2; Table 2). This model included age, sex, toothbrushing frequency, regular dental visit, smoking, BMI, diabetes, job, number of present teeth, and DFT. When different definitions of tooth loss were used, similar results were obtained (Table S1). The incidence of tooth loss was lower in participants with the highest quartile of yogurt product intake than those with no intake, who were matched for the frequency of toothbrushing and dental visits, smoking status, and diabetes (Table S2). This association was found after adjusting for sex and age (IRR 0.88, 95% CI 0.78–0.99). The association between yogurt

### TABLE 1  Baseline characteristic of participants according to yogurt product intake

| Quartile of yogurt product intake, g/day | All (n = 1469) | Q1 (0) (n = 395) | Q2 (0.1–28.6) (n = 314) | Q3 (28.7–79.9) (n = 389) | Q4 (≥80.0) (n = 371) | p-Value for trend |
|------------------------------------------|----------------|-----------------|-------------------------|-------------------------|---------------------|-----------------|
| Age, years (mean ± SD)                   | 59.3 ± 10.3    | 59.2 ± 10.4     | 57.4 ± 10.4             | 59.8 ± 10.2             | 60.4 ± 10.2         | .019            |
| Women, %                                 | 55.7           | 34.4            | 56.1                    | 67.9                    | 65.2                | <.001           |
| Toothbrushing frequency >1 time, %       | 74.5           | 60.5            | 74.5                    | 80.2                    | 83.3                | <.001           |
| Regular dental visit, %                  | 35.6           | 29.4            | 30.6                    | 36.8                    | 45.3                | <.001           |
| Current smoking, %                       | 15.3           | 28.6            | 16.6                    | 9.0                     | 6.7                 | <.001           |
| BMI, kg/m² (mean ± SD)                   | 23.2 ± 3.4     | 23.4 ± 3.3      | 23.3 ± 3.7              | 23.0 ± 3.4              | 23.0 ± 3.3          | .041            |
| Diabetes, %                              | 13.8           | 14.2            | 15.6                    | 12.9                    | 12.9                | <.001           |
| Number of teeth present, (mean ± SD)     | 25.4 ± 4.1     | 25.4 ± 4.3      | 25.6 ± 3.8              | 25.1 ± 4.5              | 25.6 ± 3.8          | .544            |
| Number of DFT, (mean ± SD)               | 14.6 ± 5.3     | 13.8 ± 5.6      | 14.8 ± 5.0              | 14.9 ± 5.1              | 14.9 ± 5.3          | .004            |
| Mean CAL, (mean ± SD)                    | 2.22 ± 0.78    | 2.46 ± 0.91     | 2.20 ± 0.82             | 2.10 ± .65              | 2.11 ± 0.66         | <.001           |
| Mean PD, (mean ± SD)                     | 2.01 ± 0.67    | 1.91 ± 0.59     | 1.83 ± 0.59             | 1.78 ± 0.50             | 1.70 ± 0.47         | <.001           |
| %BOP, (mean ± SD)                        | 11.3 ± 15.3    | 18.1 ± 21.3     | 17.3 ± 21.4             | 14.3 ± 17.8             | 11.6 ± 15.2         | <.001           |

Note: The p-value for trend was calculated from linear and logistic regressions using the quartile ordinal as the predictor variable. Abbreviations: BMI, body mass index; BOP, bleeding on probing; CAL, clinical attachment level; DFT, decayed and filled teeth; PD, pocket depth.

### TABLE 2  Incidence rate ratios for tooth loss according to yogurt product intake

| Quartile of yogurt product intake | Incidence of tooth loss | IRR (95% CI) for tooth loss |
|----------------------------------|------------------------|----------------------------|
|                                  |                        | Model 1 | Model 2 | Model 3 |
| Q1 (low)                         | 23.3                   | 1       | 1       | 1       |
| Q2                               | 17.2                   | 0.85 (0.63–1.14) | 0.85 (0.64–1.14) | 0.90 (0.68–1.19) |
| Q3                               | 15.7                   | 0.71 (0.53–0.95) | 0.73 (0.55–0.96) | 0.78 (0.59–1.03) |
| Q4 (high)                        | 14.3                   | 0.63 (0.46–0.85) | 0.73 (0.53–0.99) | 0.76 (0.56–1.02) |
| p-Value for trend                | .001                   | .020    | .067    |
| Number of DFT                    | 1.03 (1.01–1.05)       |        |        |        |
| Mean CAL                         | 1.43 (1.27–1.61)       |        |        |        |

Note: Poisson regression models; tooth loss was the dependent variable and yogurt product intake was the independent variable. Model 1 included age and sex. Model 2 included age, sex, toothbrushing frequency, regular dental visit, smoking, BMI, diabetes, job, number of teeth present, and DFT. Model 3 included variables in model 2 plus mean CAL. Abbreviations: BMI, body mass index; CAL, clinical attachment level; CI, confidence interval; DFT, decayed and filled teeth; IRR, incidence rate ratio.
Mediation analysis (a) with number of DFT as a mediator or (b) with mean CAL as a mediator of association with yogurt product intake and tooth loss

|                        | OR (95% CI) | p-Value |
|------------------------|------------|---------|
| (a) Number of DFT as a mediator |            |         |
| Direct effect (yogurt product intake → tooth loss) | 0.86 (0.74–0.97) | .018    |
| Indirect effect (yogurt product intake → DFT → tooth loss) | 1.00 (0.99–1.01) | .699    |
| Proportion of total effect mediated | –1.5%     |         |
| (b) Mean CAL as mediator |            |         |
| Direct effect (yogurt product intake → tooth loss) | 0.89 (0.77–1.02) | .089    |
| Indirect effect (yogurt product intake → CAL → tooth loss) | 0.97 (0.95–0.99) | .008    |
| The proportion of total effect mediated | 19.7%      |         |

Note: Adjusted for age, sex, toothbrushing frequency, regular dental visit, smoking, BMI, diabetes, job, and the number of present teeth. Abbreviations: BMI, body mass index; CAL, clinical attachment level; CI, confidence interval; DFT, decayed and filled teeth; OR, odds ratio.

TABLE 3 Mediation analysis (a) with number of DFT as a mediator or (b) with mean CAL as a mediator of association with yogurt product intake and tooth loss

The number of DFT as a mediator

Indirect effect (yogurt product intake → tooth loss) 1.00 (0.99–1.01) .699

Proportion of total effect mediated –1.5%

(a) Number of DFT as a mediator

(b) Mean CAL as mediator

Direct effect (yogurt product intake → tooth loss) 0.89 (0.77–1.02) .089

Indirect effect (yogurt product intake → CAL → tooth loss) 0.97 (0.95–0.99) .008

The proportion of total effect mediated 19.7%

Note: Adjusted for age, sex, toothbrushing frequency, regular dental visit, smoking, BMI, diabetes, job, and the number of present teeth. Abbreviations: BMI, body mass index; CAL, clinical attachment level; CI, confidence interval; DFT, decayed and filled teeth; OR, odds ratio.

4 | DISCUSSION

The primary finding of this study was the negative association between the intake of yogurt products and tooth loss over 5 years. This study extends the findings of previous cross-sectional studies (Shimazaki et al., 2008; Kim et al., 2017) regarding the association between yogurt product intake and periodontitis. Furthermore, this longitudinal association was shown to be partly mediated by periodontal conditions via the probable modulation of the oral microbiome composition.

It has been recognized that the intake of dairy products has health benefits such as a reduced risk of diabetes, metabolic syndrome, and cardiovascular disease (Pfeffer & Schrezenmeir, 2007). Previous studies have reported that individuals who consume high quantities of dairy products were less likely to have periodontitis (Al-Zahrani, 2006; Adegboye et al., 2012). Additionally, frequent intake of milk has been reported to be associated with a low prevalence of periodontitis (Adegboye et al., 2012; Lee & Kim, 2019). Milk and dairy products are good sources of calcium and other important nutrients for bone development and maintenance (Heaney, 2000). Calcium intake may have a favourable effect on oral health by enhancing the alveolar bone density (Nishida et al., 2000). However, the energy-adjusted intake of milk and calcium using the density method did not have any significant effect on tooth loss in this study (Table S3), which suggested that other constituents may have a greater impact on tooth loss in our study population. Two studies had revealed that yogurt product intake was associated with periodontitis, but milk intake was not (Shimazaki et al., 2008; Kim et al., 2017). Yogurt products have nutrients similar to those in milk; however, they are characterized by many live lactic acid bacteria that act as probiotics.

Probiotics play a role in the shift in bacterial biofilm composition, virulence, and subsequent host reactions. The potential effects of probiotic species on periodontal pathogens have been demonstrated in vitro (Järsberg et al., 2016). In this study, high consumption of yogurt products was associated with a lower percentage of participants with type I salivary microbiome (Figure 1). The type I salivary microbiome was characterized by the dominance of cohabiting bacterial groups, including Streptococcus, Rothia, Prevotella, and Veillonella species, whereas type II was characterized by the dominance of cohabiting bacterial groups including Neisseria and Porphyromonas species (Figure S2). Our previous study had revealed that the type I salivary microbiome is associated not only with poor oral health conditions (Takeshita et al., 2016; Zhang et al., 2021) but also with a high production of acetaldehyde in the saliva (Yokoyama et al., 2018) and health

FIGURE 1 Age- and sex-adjusted percentage of salivary microbiome community type I according to yogurt product intake. After excluding individuals whose saliva sample could not be collected (n = 176), the quartiles of yogurt product intake are 0, 0.1–29.2, 29.3–80.0, >80.0 g/day in 1293 participants. The p-value for trend was calculated using the Poisson regression model using the quartile ordinal as the predictor variable, adjusted for age, sex, toothbrushing frequency, regular dental visit, body mass index, and diabetes

products and tooth loss was not significant in Model 3, including mean CAL (Table 2).

Mediation analysis showed that the association between yogurt product intake and tooth loss was partially mediated by the mean CAL (19.7%) (Table 3). No indirect association of yogurt product intake with tooth loss through the number of DFT was found. As the indirect effect and total effect were in opposing directions, the model in the number of DFT had a negative mediated proportion.

While 40.9% of the total participants had a type I salivary microbiome, the percentage was significantly lower in those in the highest quartile of yogurt product intake when adjusted for various confounding factors (Figure 1).
problems related to pneumonia in elderly residents nursed at home (Takeshita et al., 2010; Kageyama et al., 2018) even in different populations. It has been reported that the genera Prevotella and Veillonella significantly increased with a concurrent decrease in the genus Neisseria in the salivary microbiota of patients with inflammatory bowel disease whose saliva showed elevated levels of many inflammatory cytokines and immunoglobulin A and a lower lysozyme level (Said et al., 2014). Prevotella and Veillonella species predominate in type I salivary microbiome, while Neisseria species predominate in type II salivary microbiome. Individuals with type I salivary microbiome had fewer teeth present, more dental caries, and poor periodontal condition compared to those with type II microbiome (Takeshita et al., 2016). In this study, we found that type I salivary microbiome was positively associated with tooth loss (Table S4). Lactic acid bacteria from yogurt products may lead to a shift in the bacterial composition, which is related to good oral health conditions.

We conducted three separate analyses to evaluate the potential pathway from yogurt product intake to tooth loss: (1) the association between yogurt product intake and tooth loss was mediated by periodontal condition (Table 3); (2) yogurt product intake was associated with the salivary microbiome type (Figure 1); and (3) the salivary microbiome type was associated with tooth loss (Table S4). Subsequently, we tested the simultaneous regression models using path analysis (Figure S3). This analysis is a technique that allows an understanding of the complex inter-relationships among multiple variables and testing the causal model. Although data on yogurt product intake, periodontal condition, and salivary microbiome type were cross-sectional, we proposed a plausible pathway based on the results of path analysis: yogurt product intake leads to alterations in the oral microbiome and improves the periodontal condition, which indirectly contributes to reduced tooth loss. When we made an analysis using the data on periodontal conditions at follow-up, we confirmed that high intake of yogurt products was associated with a low mean value of CAL, PPD, and %BOP at follow-up (Table S5). This finding suggests that yogurt product intake contributes to good periodontal health.

This study had several limitations. First, the reasons for tooth extraction (dental caries or periodontal disease) were not investigated owing to the limited examination time. Second, this study included participants who had periodontal disease and dental caries. Ideally, participants without these diseases at baseline should be followed up to evaluate the independent association between yogurt product intake and tooth loss. However, the influence of these diseases was adjusted for in the multivariate Poisson regression model and mediation analysis in this study to secure the sample size. Third, oral-disease-related factors, such as the use of interdental brushes and fluoride toothpaste, were not assessed in this study. Future studies are required to evaluate these factors. On the one hand, mediation analysis showed no indirect association between yogurt product intake and tooth loss through dental caries. The use of fluoride toothpaste seemed to be less likely to affect the association between yogurt product intake and tooth loss. Fourth, factors such as the socio-economic status of the participants may affect the dentist's decision to extract a tooth. This may cause a bias in the observed association between the risk factors and tooth loss. However, the Japanese public health insurance system covers almost all dental therapies; therefore, socio-economic status may have little effect in Japan. Fifth, the consumption of yogurt products is considered to be a signature of a healthy diet (Panahi et al., 2017). Its consumption is more common in healthier, leaner, and more highly educated individuals, and is more widespread in women (Tremblay & Panahi, 2017). In our study, consumers of high yogurt products were characterized by higher levels of oral health behaviour and healthier conditions (Table 1). Even after adjustment for these factors, the findings might have been biased by residual confounding factors. Finally, we used a partial-mouth assessment for periodontal conditions, which did not include an examination of the lingual or palatal sites. Our results potentially underestimate periodontal conditions.

In conclusion, this study demonstrated that a higher intake of yogurt products was a potentially protective factor against future tooth loss, by avoiding deterioration of the periodontal condition. The oral microbiome is considered to play a role in this association. Our results suggest that the intake of yogurt products is recommended to prevent tooth loss.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Jiale Ma, Michiko Furuta, Kazuhiro Uchida, and Yoshihisa Yamashita conceived and designed the study. Jiale Ma, Michiko Furuta, Toru Takeshita, and Shinya Kageyama conducted the statistical analyses. Jiale Ma, Michiko Furuta, Kazuhiro Uchida, Toru Takeshita, Woosung Sohn, Jun Hata, and Yoshihisa Yamashita interpreted the data and drafted the manuscript. Jiale Ma, Michiko Furuta, Kazuhiro Uchida, Toru Takeshita, Shinya Kageyama, Mikari Asakawa, Kenji Takeuchi, Shino Suma, Satoko Sakata, Jun Hata, Yoshiharu Ninomiya, and Yoshihisa Yamashita acquired the data. All authors gave their final approval and agreed to be accountable for all aspects of the work.

ETHICAL STATEMENT

This study was approved by the Kyushu University Institutional Review Board for Clinical Research (Approval No. 28-31).

DATA AVAILABILITY STATEMENT

The data are not publicly available due to ethical restriction.

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