Table 1. Characteristics of male participants by masticatory performance.

	Masticatory performance				
	Q1 (lowest)	Q2	Q3	Q4 (highest)	*P* trend
Participants, *n*	572	570	571	570	
Age, *y*	65.0 (59.0–69.0)	63.0 (56.0–68.0)	62.0 (54.0–67.0)	61.0 (54.0–67.0)	<0.001
Height, *cm*	166.6 (162.4–170.9)	167.1 (163.0–171.2)	167.2 (163.3–171.7)	167.9 (163.7–172.1)	<0.001
Weight, *kg*	64.1 (57.8–71.0)	64.7 (58.7–70.7)	65.6 (59.6–71.6)	65.2 (60.3–72.7)	<0.001
Body mass index, kg/m^2	23.1 (21.2–25.0)	23.2 (21.1–25.1)	23.3 (21.7–25.1)	23.5 (21.5–25.2)	0.018
Blood pressure, *mm Hg*					
Systolic	128.5 (120.0–141.0)	127.0 (118.0–138.0)	128.0 (119.0–139.0)	129.0 (119.0–140.0)	0.94
Diastolic	80.0 (73.0–87.0)	80.0 (74.0–87.0)	80.0 (73.0–88.0)	82.0 (75.0–89.0)	0.004
HbA1c, %	5.6 (5.3–5.9)	5.5 (5.3–5.8)	5.5 (5.3–5.8)	5.5 (5.3–5.8)	0.075
Glucose level, *mg/dl*	92.0 (86.0–101.0)	91.0 (85.0–98.0)	91.0 (85.0–97.0)	91.0 (86.0–98.0)	0.097
Total cholesterol, *mg/dl*	200.0 (177.0–221.0)	197.5 (179.0–222.0)	203.0 (182.0–222.0)	203.0 (182.0–229.0)	0.003
High-density cholesterol, *mg/dl*	54.0 (45.0–65.0)	55.0 (45.0–67.0)	56.0 (47.0–69.0)	57.0 (48.0–67.0)	0.002
Triglyceride, *mg/dl*	105.0 (74.0–149.5)	100.5 (70.0–140.0)	102.0 (74.0–146.0)	101.0 (72.0–143.0)	0.53
Prevalence of diabetes, *n* (%)	57 (9.9)	48 (8.4)	42 (7.3)	30 (5.2)	0.002
Family history of diabetes, *n* (%)					
No	466 (81.4)	441 (77.3)	450 (78.8)	460 (80.7)	0.90
Yes	106 (18.5)	129 (22.6)	121 (21.1)	110 (19.3)	
Smoking, *n* (%)					
Never	90 (15.7)	128 (22.4)	144 (25.2)	170 (29.8)	<0.001
Former	258 (45.1)	273 (47.8)	310 (54.2)	283 (49.6)	
Current	224 (39.1)	169 (29.6)	117 (20.4)	117 (20.5)	
Alcohol drinking, *n* (%)					
Never	97 (16.9)	75 (13.1)	73 (12.7)	58 (10.1)	0.001
Former	21 (3.6)	19 (3.3)	16 (2.8)	17 (2.9)	
Current	454 (79.3)	476 (83.5)	482 (84.4)	495 (86.8)	
Physical activity, *n* (%)					
Slight	61 (10.6)	75 (13.1)	56 (9.8)	56 (9.8)	0.86
Moderate	344 (60.1)	347 (60.8)	349 (61.1)	359 (62.9)	
Strenuous	167 (29.2)	148 (25.9)	166 (29.0)	155 (27.1)	
Caloric restriction, *n* (%)					
No	378 (66.0)	378 (66.3)	382 (66.9)	398 (69.8)	0.28
Yes, at subject discretion	168 (29.3)	164 (28.7)	153 (26.8)	139 (24.3)	
Yes, due to medical diagnosis	26 (4.5)	28 (4.9)	36 (6.3)	33 (5.7)	
Rate of eating, *n* (%)					
Slow	61 (10.6)	55 (9.6)	52 (9.1)	37 (6.4)	0.006
Intermediate	313 (54.7)	302 (52.9)	295 (51.6)	301 (52.8)	
Fast	198 (34.6)	213 (37.3)	224 (39.2)	232 (40.7)	
Periodontal status, *n* (%)					
Healthy	75 (13.1)	54 (9.4)	80 (14.0)	64 (11.2)	<0.001
Gingival bleeding	16 (2.8)	35 (6.1)	56 (9.8)	46 (8.0)	
Supra- or sub-gingival calculus	59 (10.3)	74 (12.9)	81 (14.1)	113 (19.8)	
Shallow periodontal pockets	145 (25.3)	177 (31.0)	169 (29.6)	169 (29.6)	
Deep periodontal pockets	220 (38.4)	216 (37.8)	180 (31.5)	176 (30.8)	
Edentulous	57 (9.9)	14 (2.4)	5 (0.8)	2 (0.3)	

Continuous variables are presented as medians (interquartile range).
Categorical variables are presented as numbers (%).
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On the other hand, a few studies have demonstrated direct relationships between mastication and glucose metabolism. Thorough mastication elicited a lower postprandial plasma glucose concentration because of the potentiation of early-phase insulin secretion [13]. Eating slowly lead to lower postprandial concentrations of the anorexigenic gut peptides peptide YY and glucagon-like peptide 1 (GLP-1) [14,15]. These findings indicate that adequate eating habits prevent the incidence of diabetes by improving glucose metabolism after meals.

Recently, a few studies have reported an association between the rate of eating and the risk of diabetes [16,17]. To date, however, the association between mastication, particularly masticatory performance, and diabetes has not been clarified.

Here, we investigated the association between mastication, namely masticatory performance or rate of eating, and diabetes in a population-based cohort.

Methods

Study design and population

We conducted a cross-sectional study of the association between masticatory performance and diabetes. Subjects were participants in the Nagahama Prospective Genome Cohort for Comprehensive Human Bioscience (the Nagahama Study) [18]. The Nagahama Study is a longitudinal genetic epidemiological study aimed at clarifying as-yet unidentified factors and pathways relating genetic variants and disease phenotypes of common diseases and disorders, such as cardiovascular diseases, endocrine and metabolic diseases, immunological diseases and oral diseases via the comprehensive analysis of omics data. The Nagahama Study participants were recruited from apparently healthy community residents aged 30 to 74 years living in Nagahama City, a largely rural city of approximately 125,000 inhabitants in Shiga Prefecture, located in the center of Japan. A total of 9,804 participants were recruited during 2008 to 2010. Among 8,679 participants who underwent oral examination and masticatory performance tests in 2009–2010, inclusion in the present study was restricted to participants aged 40 years or older who completed all baseline measurements, and participants who were pregnant or who had a history of gestational diabetes were excluded. After applying these eligibility criteria, a total of 6,827 participants were entered into the analysis.

Ethics statement

This study protocol was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine, the Ethical Review Board of the Nagahama Study, and the Nagahama Municipal Review Board of Personal Information Protection. Written informed consent was obtained from all participants.

Measurement of masticatory performance

Participants were instructed to masticate a piece of chewing gum (Lotte Co., Ltd.) as usual for one minute. Participants who had a denture were instructed to wear it and masticate. The chewing gum changes color as mastication proceeds. After each trial, the chewed gum bolus was placed between two plastic films and pressed into an approximately 30-mm diameter disk. Color measurement using a CR-13 spectrophotometer (Konica Minolta Holdings, Inc.) was performed through the plastic films at five sites, one in the center and four others in the midpoint of imaginary spoke lines extending from the center to the superior, inferior, left, and right margins on the surface of the flattened gum [19].

Measurement was performed by an experienced dentist. Color was evaluated using the L*a*b* color space, which was developed by the Commission Internationale de l'Eclairage (CIE) for measuring object color [20]. Mean values of L*a*b* measured at the five sites on the gum were used to estimate the color difference ΔE^*ab, which is calculated by the following equation: $\Delta E^*ab = \{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\}^{1/2}$ [20]. Respective values of L*a*b* before chewing were 73.1, −11.6, and 34.4 (means). We regarded the estimated ΔE^*ab as the masticatory performance of the participant [21,22]. We then divided participants into four groups by quartile of masticatory performance.

Definition of diabetes

The value for HbA1c (%) was estimated as an National Glycohemoglobin Standardization Program (NGSP)-equivalent value (%), calculated by the formula A1C (%) = A1C (Japan Diabetes Society (JDS)) (%) + 0.4%, in consideration of the relational expression of HbA1c (JDS) (%) measured by the previous Japanese standard substance and measurement methods and A1C (NGSP) [23]. Participants were considered diabetic if they met at least one of the following parameters: fasting blood glucose level ≥126 mg/dl (≥7.0 mmol/l), random plasma glucose level ≥200 mg/dl (≥11.1 mmol/l), or HbA1c ≥6.5% (HbA1c ≥6.1% according to JDS) [23]. Diabetes was diagnosed if the blood sample was confirmed to be a diabetic type both by plasma glucose level and HbA1c at the same time [23], or the participant had received any treatment with hypoglycemic medication (hypoglycemic agent and/or insulin). Fasting was defined as no caloric intake for at least 8 hours [24].

Dependent variables

The health examination included height, weight, blood pressure, and blood tests. Two sitting blood pressure measurements were averaged for analysis. Blood samples from each participant were used to measure total cholesterol, high-density cholesterol, triglyceride, plasma glucose, and HbA1c. In addition, oral examinations were conducted by two trained dentists, with an inter-examiner reliability of 0.77 to 1.0 by kappa statistics. Oral examination included DMF index and periodontal status, excluding third molars. The DMF index, which comprises the number of decayed (D), missing (M), and filled (F) teeth, has been established as a key measurement of caries experience in dental epidemiology [25]. We then estimated the number of present teeth as 28 − the number of missing teeth. Periodontal status was assessed according to the Community Periodontal Index (CPI) [25]. CPI was used to assess the presence or absence of periodontal disease in each sextant (i.e. sixth of dentition) according to the following items: (Score 0) healthy, (Score 1) gingival bleeding after probing, (Score 2) supra- or sub-gingival calculus, (Score 3) pocket depth between 4 and 5 mm and, (Score 4) pocket depth between 6 mm or more. We used a standardized lightweight periodontal probe with a 0.5-mm ball tip to probe standardized index teeth and categorized periodontal status with a score of 0 to 4. Index teeth were investigated as the recommended 10 teeth; if the index tooth was missing, the next adjacent tooth was used for evaluation [26]. Subjects with complete edentulousness were entered into a separate category for the calculation of CPI [26].

A self-administered questionnaire was used to assess medical history, including history of diabetes, type of hypoglycemic medication, and history of diabetes in a first-degree relative (no or yes). In addition, life-style variables were surveyed, including smoking habit (never, former, or current), alcohol drinking habit (never, former, or current), physical activity (slight, moderate, or strenuous), caloric restriction (no, yes at subject discretion, or yes

Table 2. Characteristics of female participants by masticatory performance.

	Masticatory performance				
	Q1 (lowest)	Q2	Q3	Q4 (highest)	*P* trend
Participants, *n*	1,139	1,136	1,133	1,136	
Age, *y*	61.0 (54.0–67.0)	59.0 (50.5–65.0)	59.0 (50.0–64.0)	59.0 (52.0–65.0)	<0.001
Height, *cm*	154.3 (150.4–158.2)	155.2 (151.1–159.1)	155.5 (151.7–159.2)	155.1 (151.1–159.1)	<0.001
Weight, *kg*	52.6 (47.4–57.9)	52.1 (48.1–57.4)	52.1 (47.7–57.4)	52.5 (48.1–57.6)	0.58
Body mass index, kg/m^2	22.0 (20.2–24.2)	21.7 (19.9–23.8)	21.4 (19.8–23.7)	21.9 (20.1–23.9)	0.18
Blood pressure, *mm Hg*					
Systolic	123.0 (112.0–134.0)	120.0 (109.0–131.0)	121.0 (110.0–132.0)	122.0 (111.0–133.0)	0.15
Diastolic	76.0 (68.0–83.0)	74.0 (67.0–81.0)	74.0 (68.0–82.0)	75.0 (68.0–82.0)	0.83
HbA1c, %	5.5 (5.3–5.7)	5.5 (5.3–5.7)	5.5 (5.3–5.7)	5.5 (5.3–5.7)	0.12
Glucose level, *mg/dl*	89.0 (84.0–93.0)	88.0 (83.0–93.0)	88.0 (84.0–93.0)	88.0 (84.0–93.0)	0.58
Total cholesterol, *mg/dl*	215.0 (193.0–237.0)	213.0 (192.0–234.0)	215.0 (192.0–238.0)	216.0 (197.0–239.0)	0.036
High-density cholesterol, *mg/dl*	65.0 (55.0–77.0)	67.0 (56.0–78.0)	68.0 (57.0–80.0)	69.0 (57.0–81.0)	<0.001
Triglyceride, *mg/dl*	84.0 (61.0–118.0)	78.0 (60.0–112.5)	77.0 (57.0–108.0)	79.0 (59.0–109.5)	0.001
Prevalence of diabetes, *n* (%)	33 (2.9)	34 (2.9)	28 (2.4)	17 (1.5)	0.022
Family history of diabetes, *n* (%)					
No	886 (77.7)	860 (75.7)	852 (75.2)	859 (75.6)	0.21
Yes	253 (22.2)	276 (24.3)	281 (24.8)	277 (24.3)	
Smoking, *n* (%)					
Never	1,002 (87.9)	1,024 (90.1)	1,029 (90.8)	1,044 (91.9)	0.001
Former	57 (5.0)	68 (5.9)	69 (6.0)	59 (5.1)	
Current	80 (7.0)	44 (3.8)	35 (3.0)	33 (2.9)	
Alcohol drinking, *n* (%)					
Never	619 (54.3)	554 (48.7)	545 (48.1)	565 (49.7)	0.015
Former	21 (1.8)	6 (0.5)	11 (0.9)	7 (0.6)	
Current	499 (43.8)	576 (50.7)	577 (50.9)	564 (49.6)	
Physical activity, *n* (%)					
Slight	64 (5.6)	84 (7.3)	67 (5.9)	68 (5.9)	0.76
Moderate	856 (75.1)	816 (71.8)	842 (74.3)	840 (73.9)	
Strenuous	219 (19.2)	236 (20.7)	224 (19.7)	228 (20.0)	
Caloric restriction, *n* (%)					
No	633 (55.5)	636 (55.9)	575 (50.7)	601 (52.9)	0.090
Yes, at subject discretion	451 (39.6)	455 (40.0)	500 (44.1)	495 (43.5)	
Yes, due to medical diagnosis	55 (4.8)	45 (3.9)	58 (5.1)	40 (3.5)	
Rate of eating, *n* (%)					
Slow	125 (10.9)	89 (7.8)	120 (10.5)	84 (7.3)	0.025
Intermediate	678 (59.5)	684 (60.2)	652 (57.5)	677 (59.6)	
Fast	336 (29.5)	363 (31.9)	361 (31.8)	375 (33.0)	
Periodontal status, *n* (%)					
Healthy	230 (20.1)	197 (17.3)	206 (18.1)	192 (16.9)	<0.001
Gingival bleeding	110 (9.6)	162 (14.2)	148 (13.0)	141 (12.4)	
Supra- or sub-gingival calculus	150 (13.1)	171 (15.0)	217 (19.1)	248 (21.8)	
Shallow periodontal pockets	333 (29.2)	378 (33.2)	352 (31.0)	351 (30.9)	
Deep periodontal pockets	276 (24.2)	220 (19.3)	207 (18.2)	203 (17.8)	
Edentulous	40 (3.5)	8 (0.7)	3 (0.2)	1 (0.1)	

Continuous variables are presented as medians (interquartile range).
Categorical variables are presented as numbers (%).
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Table 3. Masticatory performance and risk of diabetes.

	Masticatory performance				
	Q1 (lowest)	Q2	Q3	Q4 (highest)	*P* trend
Male					
Number of subjects with diabetes	57	48	42	30	
Number of subjects without diabetes	515	522	529	540	
Crude (95% CI)	1.0 (ref)	0.83 (0.55–1.2)	0.71 (0.47–1.0)	0.50 (0.31–0.79)	0.003
Age-adjusted OR (95% CI)	1.0 (ref)	0.89 (0.59–1.3)	0.77 (0.50–1.1)	0.55 (0.34–0.87)	0.015
Multivariable-adjusted OR (95% CI)	1.0 (ref)	0.91 (0.58–1.4)	0.77 (0.48–1.2)	0.53 (0.31–0.90)	0.031
Female					
Number of subjects with diabetes	33	34	28	17	
Number of subjects without diabetes	1,106	1,102	1,105	1,119	
Crude (95% CI)	1.0 (ref)	1.0 (0.63–1.6)	0.84 (0.50–1.4)	0.50 (0.28–0.91)	0.028
Age-adjusted OR (95% CI)	1.0 (ref)	1.1 (0.70–1.8)	0.96 (0.57–1.6)	0.55 (0.30–0.99)	0.074
Multivariable-adjusted OR (95% CI)	1.0 (ref)	1.2 (0.73–2.0)	0.95 (0.54–1.6)	0.56 (0.30–1.0)	0.083

OR = odds ratio; CI = confidence interval; ref = reference.
Multivariate ORs for diabetes were adjusted for age (40–49, 50–64 or 65–74), body mass index (<18.5, 18.5–24.9, 25.0–29.9 or ≥30.0), family history of diabetes (no or yes), current smoking (no or yes), current alcohol drinking (no or yes), physical activity (slight, moderate, or strenuous), caloric restriction (no, yes at subject discretion, or yes due to medical diagnosis), rate of eating (slow, intermediate, or fast) and periodontal status (no pathological pockets (score 0, 1 and 2), periodontal pockets (score 3 and 4), or edentulous).
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due to medical diagnosis) and rate of eating (slow, intermediate, or fast). We also surveyed the intake of 18 kinds of foods, including rice, meat, fish, tofu (soybean curd), eggs, milk, and vegetables using a self-answered questionnaire, with categories of every day, 4–5 times per week, 2–3 times per week, and 1 time or less per week.

Statistical analysis

All analyses were stratified by sex. A nonparametric test for trend across ordered groups were performed. Spearman correlation coefficients were used to assess the association between masticatory performance and the other continuous variables. To adjust for demographic and possible confounding factors, logistic regression analysis was performed with diabetes as a dependent variable, and the odds ratio (OR) of diabetes and 95% confidence interval (CI) were estimated in three models: Model 1, crude; Model 2, adjusted for age (40–49, 50–64, 65–74); and Model 3, adjusted for model 2 and other possible confounding factors. In the selection of variables, we used a forced entry method and entered the following variables into the model as possible

Table 4. Rate of eating and risk of diabetes.

	Rate of eating			
	Fast	Intermediate	Slow	*P* trend
Male				
Number of subjects with diabetes	76	94	7	
Number of subjects without diabetes	791	1,117	198	
Crude (95% CI)	1.0 (ref)	0.87 (0.63–1.2)	0.36 (0.16–0.81)	0.026
Age-adjusted OR (95% CI)	1.0 (ref)	0.77 (0.56–1.0)	0.30 (0.13–0.67)	0.002
Multivariable-adjusted OR (95% CI)	1.0 (ref)	0.87 (0.61–1.2)	0.38 (0.16–0.91)	0.048
Female				
Number of subjects with diabetes	39	61	12	
Number of subjects without diabetes	1,396	2,630	406	
Crude (95% CI)	1.0 (ref)	0.83 (0.55–1.2)	1.0 (0.54–2.0)	0.75
Age-adjusted OR (95% CI)	1.0 (ref)	0.79 (0.52–1.1)	0.96 (0.49–1.8)	0.50
Multivariable-adjusted OR (95% CI)	1.0 (ref)	0.92 (0.59–1.4)	1.5 (0.73–3.0)	0.65

OR = odds ratio; CI = confidence interval; ref = reference.
Multivariate ORs for diabetes were adjusted for age (40–49, 50–64, or 65–74), body mass index (<18.5, 18.5–24.9, 25.0–29.9, or ≥30.0), family history of diabetes (no or yes), current smoking (no or yes), current alcohol drinking (no or yes), physical activity (slight, moderate, or strenuous), caloric restriction (no, yes at subject discretion, or yes due to medical diagnosis), masticatory performance (Q1, Q2, Q3 or Q4) and periodontal status (no pathological pockets (score 0, 1 and 2), periodontal pockets (score 3 and 4), or edentulous).
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confounding factors: age, body mass index (BMI) (<18.5, 18.5–24.9, 25.0–29.9 or ≥30.0), family history of diabetes (no or yes), current smoking (no or yes), current alcohol drinking (no or yes), physical activity (slight, moderate or strenuous), caloric restriction (no, yes at subject discretion, or yes due to medical diagnosis), rate of eating (slow, intermediate, or fast) and periodontal status (no pathological pockets (score 0, 1 and 2), periodontal pockets (score 3 and 4), or edentulous). Goodness of fit of the model was examined using the Hosmer-Lemeshow goodness of fit test. Linear tests for trend were performed with the median value in each group as a continuous variable [27]. All P values were two sided at a significance level of 5%. All statistical analyses were performed using Stata 11.2 software (Stata Corporation, College Station, TX, USA).

Results

The distribution of masticatory performance and prevalence of diabetes in the participants are shown in Appendix S1. Masticatory performance was lower and the prevalence of diabetes was higher with age in both males and females. Large differences in the prevalence of diabetes between males and females were seen for all age groups, with an average prevalence of 7.7% in males, 2.4% in females, and 4.2% in all.

Masticatory performance was divided into quartiles, namely quartiles one (1.85 to 34.21), two (34.22 to 40.45), three (40.47 to 46.12) and four (46.14 to 59.9) in males, and one (2.63 to 32.44), two (32.45 to 38.21), three (38.22 to 43.05) and four (43.06 to 56.15) in females. We divided participants into four groups according to quartile of masticatory performance, namely Q1 (lowest), 2, and 3 and 4 (highest) groups. Characteristics of males and females by masticatory performance are shown in Tables 1 and 2. A positive correlation between masticatory performance and height was seen in both males and females. Similar correlations were seen in weight and BMI in males, but not in females. Regarding lifestyle, the rate of smokers (former and current) was lower as masticatory performance increased in both males and females. In addition, the rate of participants eating fast was higher and the prevalence of periodontitis was lower. In contrast, no large differences were found among the four groups regardless of sex in blood pressure, family history of diabetes, physical activity, or caloric restriction.

We investigated the association between masticatory performance and prevalence of diabetes and found an inverse dose-dependent association (Table 3). Compared to the lowest group as a reference, crude odds ratio of diabetes was 0.83 (95% CI, 0.55–1.2) in Q2, 0.71 (95% CI, 0.47–1.0) in Q3 and 0.50 (95% CI, 0.31–0.79) in the high group in males, and 1.0 (95% CI, 0.63–1.6) in Q2, 0.84 (95% CI, 0.50–1.4) in Q3 and 0.50 (95% CI, 0.28–0.91) in the high group in females. These trends were still observed in males after adjustment for age (P for trend = 0.015) and for demographic and possible confounding factors (P for trend = 0.031). The multivariable adjusted OR of diabetes was 0.91 (95% CI, 0.58–1.4) in Q2, 0.77 (95% CI, 0.48–1.2) in Q3 and 0.53 (95% CI, 0.31–0.90) in the high group. In contrast, we found no significant association between masticatory performance and diabetes in females. The final multivariable adjusted model was reliable (P = 0.62 in males and P = 0.70 in females by the Hosmer–Lemeshow test).

We also conducted an additional analysis and estimated OR of diabetes by the rate of eating (Table 4). As with masticatory performance, we found that slow eating was significantly associated with decreased odds for diabetes in multivariable adjusted ORs in males (P for trend = 0.048). In contrast, we found no significant association in females.

Discussion

We examined the association between mastication and diabetes in a population-based cohort. An inverse dose-dependent association was observed between masticatory performance and diabetes in both males and females in the estimation of crude odds ratio. The association was maintained in males after adjustment for potential confounding factors. In addition, slow eating was significantly associated with decreased odds for diabetes in males. In females, in contrast, no associations were found after adjustment, albeit that this might have been due to the low prevalence of diabetes in females in our study population. To our knowledge, this is the first study to clarify the association between mastication, namely masticatory performance or rate of eating, and diabetes.

We hypothesize two possible mechanisms underlying the association between masticatory performance and diabetes. The first involves the reduced intake of nutrients such as dietary fiber or magnesium, which were lower in subjects who were unable to fully masticate due to teeth loss, ill-fitting dentures or edentulousness [10–12]. Indeed, insufficient dietary fiber, magnesium or calcium intakes were reported to be associated with the risk of type 2 diabetes [3,4,28–31]. In particular, the intake of dietary fiber reduces glucose and influences insulin responses as a result of the retarding effect of soluble fiber on gastric emptying and absorption [32]. The other mechanism underlying the association involves the habitual chewing of hard food. A hard gum chewing exercise was effective in increasing maximum bite force and masticatory performance, and the effects were maintained after exercise completion [33]. Habitual chewing of hard foods was also reported to influence body weight loss, postprandial thermogenesis and glucose metabolism, although the mechanism of these effects remains unclear [34,35]. In addition, hardness of the habitual diet was an important environmental factor in the prevention of diabetes in a mouse model [36]. These previous studies strongly support our present findings.

Second, we found that slow eating was significantly associated with decreased odds for diabetes in males, after considering dental problems and other potential confounding factors. Recent studies have found that eating fast by self-assessed questionnaire was associated with a higher risk of diabetes in middle-aged Japanese males [16], and an increased HbA1c level in diabetic patients treated with insulin [17]. In particular, Sakurai et al. reported multivariate-adjusted hazard ratios (95% CI) of 1.00 (reference) among the slow group, versus 1.68 (0.93–3.02) among the intermediate group and 1.97 (1.10–3.55) among the fast group in a 7-year cohort [16]. These results appear similar to ours; however, their target population consisted of employee or hospital-registered patients whereas ours consisted of community residents, and oral status in their population was not evaluated. A second interesting observation was that fast eating was independently and positively associated with insulin resistance [37].

The mechanism underlying the association between rate of eating and glucose metabolism may be elucidated from the following studies. Lengthening mastication (thoroughness of mastication) was reported to elicit lower postprandial plasma glucose concentrations, because of the potentiation of early-phase insulin secretion [13]. Eating slowly also increased the postprandial response of the anorexigenic gut peptides GLP-1, which plays an important role in enhancing the glucose-stimulated insulin secretion of β-cells, and peptide YY, which regulates hunger,

satiety, and energy intake [14,15]. Moreover, several studies have shown an association between fast eating and higher BMI or weight gain [5–7,17,38]. A possible explanation of these findings is that overeating or increased energy intake in fast eaters was a result of a defect in hypothalamic neural histamine [5,39], or a lowering of satiety signals transmitted to the brain, which are triggered on nutrient ingestion by gastric distension and the release of gut factors, including cholecystokinin [40]. These reports may support the argument that eating food slowly – masticating food well – prevents obesity or insulin resistance diabetes.

Mastication or chewing serves several functions, namely the breakdown of large food particles into smaller particles suitable for gastrointestinal absorption of nutrients; and lubricating and softening food particles into a bolus conducive to swallowing, thereby facilitating gastrointestinal absorption of food particles [41]. The quality of mastication can be evaluated as masticatory performance, which is determined as the capacity to reduce the size of food particles, for example almonds, by chewing for a standardized period of time. Masticatory performance has also been determined as the number of chews necessary to render food ready for swallowing [41]. Masticatory performance in the present study was evaluated using a color-changeable chewing gum, as used in a number of other studies [19,21,22,33,42–46]. Participants were instructed to chew the gum as usual regardless of the number of chews, because the focus of our study was to evaluate their regular ability to masticate food in unit time. In addition, the method is simple and quantitative, and its validity and reliability have been confirmed [44–46], with correlation coefficients for intra- and inter-examiner consistency of more than 0.88 for three different groups (dentists, adults and elderly people) [44], and significant correlation coefficient between masticatory performance and the scores of patient satisfaction questionnaires or food questionnaires [45] or number of chewable foods [46]. Hayakawa et al. quantified the chromaticity coordinate "a" only, which represents the degree of red color, on the basis that the gum color changes from purple-blue to red as mastication proceeds [19]. In 1976, however, CIE proposed the use of ΔE^*ab for small color differences in the L*a*b* color space and for differences which result from colorant mixtures [20], and this has been supported by many researchers [21,22,47]. In addition, some participants in this population could not masticate fully and their chewed gum bolus contained colorant mixtures with red and green. We therefore decided to use ΔE^*ab to evaluate masticatory performance.

Participants who answered that they ate slowly were more common in the lower masticatory performance groups. In the multivariate analysis, in contrast, slow eating was associated with decreased odds for diabetes. This result appears contradictory. Although the speed of chewing, namely the number of chewing strokes per minute, was reported to be related with masticatory performance [21,43,44,48], the association between categorical rate of eating and masticatory performance or speed of chewing is unclear. Further investigation of the association between categorical rate of eating and masticatory performance or speed of chewing in a different population is required.

Several limitations of this study warrant mention. First, it was a cross-sectional study, and a follow-up survey is accordingly required to draw causal conclusions. For instance, diabetes may influence masticatory performance by increasing susceptibility to infections and thus the risk of periodontal disease [49], which in turn decreases masticatory performance due to teeth loss [50]. Second, total calorie intake was not investigated in this study and "caloric restriction" from a self-reported questionnaire was used as a surrogate. Instead, we surveyed the intake of 18 kinds of foods. Results showed no large differences in their distribution among the four groups by quartile of masticatory performance (data not shown). Third, we were unable to examine in detail dental prosthesis condition or type of dentition, which may be associated with masticatory performance [45,46,50]. In addition, a number of physical characteristics of participants which are involved in mastication were unclear, namely the action of the teeth, masticatory muscles, temporomandibular joint, tongue and saliva [41,51]. This lack of examination might have resulted in underestimation of the extent of masticatory performance. Fourth, socioeconomic variables such as education or income were not collected in this cohort.

In conclusion, we identified an inverse dose-dependent association between masticatory performance and diabetes in a population-based cohort. After adjustment for possible confounding factors, odds of diabetes decreased gradually as masticatory performance increased. In addition, fast eating was found to be a possible risk factor for the development of diabetes. Taken together, the present and previous results indicate that slow eating and preservation of high masticatory performance by the prevention of tooth loss or maintenance of dental prosthesis might prevent the occurrence of diabetes. These are potentially modifiable factors, and this study provides important new information for physicians and dentists concerned with the prevention of diabetes.

Supporting Information

Appendix S1 Distribution of masticatory performance and prevalence of diabetes stratified by sex and age in the Nagahama cohort.
(DOCX)

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Author Contributions

Conceived and designed the experiments: TY MY KA INA AY KT AS FM SK TN NI KB. Performed the experiments: TY MY KA INA KT FM TN. Analyzed the data: TY KA. Contributed reagents/materials/analysis tools: AS. Wrote the paper: TY MY. Contributed to the initial revision of the manuscript: FM TN. Contributed to the critical revision of the manuscript: INA AY KT AS SK NI KB. Agree with manuscript results and conclusions: TY MY KA INA AY KT AS FM SK TN NI KB.

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Risk Factors and Indices of Osteomyelitis of the Jaw in Osteoporosis Patients: Results from a Hospital-Based Cohort Study in Japan

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Abstract

Background: Several studies have reported osteomyelitis of the jaw (OMJ) as a side effect of bisphosphonates (BPs), and the risk of oral BPs has been recently clarified. However, other systemic risk factors of OMJ remain unclear. Importantly, the possibility of risk classification based on the clinical characteristics of patients has not been explored. Here, we clarified risk factors of OMJ and evaluate the predictive accuracy of risk indices in osteoporosis patients.

Methods: We performed sub-analysis using a database developed for a retrospective cohort study in patients taking medications for osteoporosis at Kyoto University Hospital. Risk indices for OMJ were constructed using logistic regression analysis, and odds ratios (OR) for OMJ cases and 95% confidence intervals (CI) were estimated. Potential risk factors included in the statistical analysis were age; sex; diabetes; use of oral BPs, corticosteroids, cancer chemotherapy, antirheumatic drugs, and biologic agents; and their interactions. Risk indices were calculated by the sum of potential risk factors of an individual patient multiplied by the regression coefficients. The discriminatory power of the risk indices was assessed by receiver operating characteristic (ROC) analysis.

Results: In analysis of all patients, oral BPs (OR: 4.98, 95% CIs: 1.94-12.75), age (OR: 1.28, 95% CI: 1.06-1.60) and sex-chemotherapy interaction (OR: 11.70, 95% CI: 1.46-93.64) were significant risk factors of OMJ. Areas under the ROC curves of these risk indices provided moderate sensitivity or specificity regardless of group (0.683 to 0.718).

Conclusions: Our data suggest that oral BP use, age, and sex-chemotherapy are predictors of OMJ in osteoporosis patients. The risk indices are moderately high, and allow the prediction of OMJ incidence.

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Introduction

Oral bisphosphonates (BPs) are useful in the treatment of various bone metabolic diseases, such as postmenopausal osteoporosis or Paget disease [1], but their use is associated with the occurrence of osteonecrosis of the jaw (ONJ), also known as osteomyelitis of the jaw (OMJ), as an adverse effect. This condition is considered refractory and causes a decrease in the quality of life in patients. Although the risk of OMJ with oral BPs was initially unclear, possibly as a result of its low incidence, recent large studies have demonstrated the incidence rate of OMJ in oral BPs users or the relative risk of oral BPs in osteoporosis patients after adjustment for potential risk factors [2-10].

Several academic associations have published position papers and guidelines which suggest that practitioners should beware of the incidence of OMJ in BPs users, particularly patients with other potential systematic risk factors for OMJ, such as diabetes, corticosteroid use and cancer chemotherapy [11-14]. Most previous studies did not clarify in detail the association between systemic factors and the incidence of OMJ [2-6,8,9], however, and the risk factors have yet to be

identified and remain speculative [15]. Only one study has shown a correlation, and this was a single correlation only and was made without adjustment for oral BP use [7]. Furthermore, the prediction of OMJ on the basis of risk factors has not been established. Thus, effective decision making based on risk assessment of OMJ in osteoporosis patients is hampered by a lack of etiological information.

Here, we investigated potential systemic risk factors for OMJ and evaluated the predictive accuracy of risk indices for OMJ using data from our hospital-based cohort study in patients with osteoporosis.

Methods

Study design and cohort

We performed sub-analysis using a database previously constructed for a retrospective cohort study conducted at Kyoto University Hospital from February 2011 to July 2012 [10]. Subjects were diagnosed with osteoporosis as specified by the 10th edition of the International Classification of Diseases (ICD-10) code at Kyoto University Hospital between November 2000 and October 2010 (Appendix S1) and prescribed osteoporosis medications approved in Japan (Appendix S2). Among these patients, analysis was limited to those aged 20 years or older who had been treated with osteoporosis medications. This criterion was based on previous findings that age at first onset of BP-related ONJ was approximately 20 years [16-18].

We then excluded patients who had the presence of primary or metastatic tumors, a history of trauma or radiation therapy in the maxillofacial region, or treatment with intravenous BPs.

Data extraction

Hospital data were extracted from the electronic medical records (EMR) using an EMR retrieval system [19]. This system retrieves electronic data for both outpatients and inpatients at Kyoto University Hospital, including demographic data, diagnosis and ICD-10 code, medications and injections, laboratory tests, radiological or pathological studies, etc. We used this system to search for patients who were diagnosed with osteoporosis, OMJ, or other diseases as specified by ICD-10 code, then reviewed patients with diseases possibly related to OMJ (Appendix S3).

Outcome measurements

Although several academic societies have stated that the hallmark of BP-related ONJ is exposed necrotic bone in the maxillofacial region that has persisted for more than 8 weeks [12,13,20], we consider it difficult to distinguish ONJ from OMJ and accordingly propose grouping cases of OMJ together with ONJ. There are two reasons for this: first, radiographic findings in infected jawbone in patients treated with BPs are similar to those in BP-induced ONJ even if necrotic bone cannot be clinically visualized [21-23]; and second, the presence of osteonecrosis is a common histopathologic finding in both BP-induced ONJ and OMJ [24]. These finding suggest that the presence of bone exposure in the oral cavity is not always caused by avascular necrosis of the jaw. Several studies or reviews have also regarded ONJ as the same as OMJ [10,25-27].

OMJ was independently reconfirmed by two trained oral and maxillofacial surgeons using proposed criteria based on findings obtained from panoramic X-ray, technetium bone scan, computed tomography, histological picture or surgery, either alone or in combination. Inter-observer agreement was moderate (kappa value = 0.64 to 0.81). Detailed information on patients and methods is reported in our previous work [10,19].

Potential risk factors

The following risk factors were included into the statistical analysis: age, sex, diabetes; use of oral BPs, corticosteroids, cancer chemotherapy, antirheumatic drugs, and biologic agents; and the interactions of potential risk factors. Diabetes was diagnosed if the patient had received a diagnosis of diabetes, and had either received any treatment with hypoglycemic medication (hypoglycemic agent and/or insulin) or had an HbA1c ≥6.5% [28]. Steroid use was defined as the receipt of any treatment with corticosteroids, and chemotherapy, antirheumatic drugs and biologic agents use as the receipt of any treatment with cancer chemotherapy, antirheumatic drugs and biologic agents.

Statistical analysis

Patient characteristics were summarized using descriptive statistics (range and percentages). The risk indices for OMJ were constructed using logistic regression analysis with OMJ as the dependent variable. Odds ratios (OR) for OMJ cases and 95% confidence intervals (CI) were estimated in all patients taking any osteoporosis medication and in the subset of oral BP users. Risk indices were calculated by the sum of the potential risk factors of an individual patient multiplied by the regression coefficients. The discriminatory power of the risk indices was assessed by receiver operating characteristic (ROC) analysis. All *P* values were two-sided at the significance level of 5%. All statistical analyses were performed using SAS Version 9.2 (SAS Institute, Cary, NC, USA).

Ethics Statement

The protocol for this study was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine and the study was conducted according to the Declaration of Helsinki. We did not obtain written informed consent because this is a retrospective study. The Ethics Committee follows the ethical guidelines for epidemiological research of the Japanese Ministry of Health, Labour and Welfare, and accordingly does not require written informed consent for these studies.

Results

Figure 1 shows a patient flowchart. A total of 7,062 patients treated with osteoporosis medications and aged 20 years or older were included. After exclusion of 29 patients with primary or metastatic tumors in the oral region and/or a history of craniofacial radiation therapy and 110 patients receiving

intravenous BPs, 6,923 (98.0%) eligible patients were entered into the analysis.

Patient characteristics are summarized in Table 1. The total number of patients prescribed oral BPs was 4,129 (59.6%), while 2,794 (40.3%) received other osteoporosis drugs. Prevalence of diabetes in our cohort was higher than in the Japanese population [29]. Steroid users accounted for approximately 60% of all patients and 70% in the subset of oral BP users.

Table 2 shows potential risk factors for OMJ in all osteoporosis patients and in those limited to oral BP users. Forty-six patients developed OMJ (0.66%, 95% CI: 0.47-0.85) among all patients, and 41 developed OMJ (0.99%, 95% CI: 0.69-1.2) among oral BPs users. In the analysis of all patients, oral BPs were shown to be a strong risk factor for OMJ (OR: 4.98, 95% CI: 1.94-12.75). Age was also a significant risk factor of OMJ (OR: 1.28, 95% CI: 1.03-1.60), whereas sex, diabetes, corticosteroids, cancer chemotherapy, antirheumatic drugs and biologic agents were not associated with the incidence of OMJ. In analysis of the interaction of two potential risk factors pairs, sex-chemotherapy showed an increased risk of OMJ (OR: 11.70, 95% CI: 1.46-93.64). Similarly, in analysis among patients limited to oral BP users, significant associations were seen for age, sex and sex-chemotherapy.

Figure 2 shows ROC curves of the risk indices for all patients and oral BPs users only in predicting the incidence of OMJ. Area under the curve was 0.683 (95% CI: 0.607- 0.760) among oral BPs users and 0.718 (95% CI: 0.648-0.789) in all patients. All curves provided only moderate sensitivity or specificity in predicting the incidence of OMJ.

Discussion

Our study had two major findings. First, we clarified three systemic risk factors for OMJ in patients with osteoporosis, namely age, oral BP use and sex-chemotherapy. In contrast, diabetes, or the use of corticosteroids, antirheumatic drugs, and biologic agents were not identified as significant risk factors of OMJ. Second, our ROC analysis of risk indices of systemic risk factors, to our knowledge the first time this has been investigated, found that ROC curve provided moderate sensitivity or specificity to allow the prediction of OMJ incidence in subsets of patients at high risk of OMJ.

Previous studies did not clarify in detail the association between potential systemic risk factors and the incidence of OMJ in osteoporosis patients nor the accuracy of prediction based on such risk factors [2-10], although they did examine the relative risk of oral BPs after adjustment for these factors. In contrast, while diabetes, corticosteroids use and cancer chemotherapy have all been suggested to be risk factors of OMJ in cancer patients [30-37], diabetes and corticosteroids use were not significant risk factors of OMJ in our cohort. This result appears contradictory. We speculate that differences in the target populations of the studies influenced systemic risk factors of OMJ; in other words, patients with osteoporosis likely received quite different treatment from cancer patients, which in turn resulted in different susceptibility to OMJ. In addition, we suspect that differences in systemic risk factors were partly due to differences among the search procedures used to identify risk factors in the various studies. In particular, almost all these factors were investigated as possible confounding factors or secondary endpoints in the studies, but given that some surveys were conducted using questionnaires, interview, or chart review, and that detailed definitions of most factors were not provided, the accuracy of some diagnoses might have been low. On the contrary, data extraction from the EMR in this study was conducted using an EMR retrieval system [19], and the confirmation of risk factors was defined by rigorous diagnostic criteria [19]. This comprehensive data extraction process and confirmation of risk factors likely improve the reliability of our results.

Our ROC analysis suggests that other risk factors contribute to the incidence of OMJ. As one such predictor, oral bacteria, are hypothesized to confer risk, given that osteomyelitis in BPs users develops only in the jawbones [14], albeit that our present and previous studies have not proved the risk of poor oral hygiene or oral bacteria at the population level. A role for genetic factors has also been hypothesized, but even recent studies of genetic factors lacked sufficient statistical power to predict the incidence of OMJ [38-40]. Further investigations to examine other predictors of the incidence of OMJ in patients with osteoporosis are required.

Nevertheless, our findings do provide relevant information to support decision-making by practitioners involved in the treatment of patients taking oral BPs. At the initiation of BP use, physicians or pharmacists may predict the risk of OMJ incidence according to the clinical characteristics of patients and consult oral specialists in advance. Dentists or oral and maxillofacial surgeons may also consider these risk factors in their care of patients using oral BPs, and the modification of practice patterns is likely useful in preventing OMJ incidence.

Several limitations of the study warrant mention. First, selection bias is inherent to single-center studies, and the present study was additionally subject to inherent referral bias toward the selection of more severe cases, given that our hospital is a lead institution in Kyoto City. Patient characteristics at our hospital might therefore differ somewhat from those at other hospitals or clinics. Second, although our estimation models adjusted for potential risk factors, including age, sex, diabetes, use of oral BPs, corticosteroids, cancer chemotherapy, antirheumatic drugs, and biologic agents and their interactions, no adjustment was made for other possible risk factors related to OMJ, such as smoking or oral BP dose, etc.[41]. However, several studies reported that there was no association between dose of oral BP or other risk factors and OMJ [5,6,9], and risk factors of BPs-related OMJ are controversial. Third, we were unable to examine in detail the primary diseases, severity of illness, and drug dosages in our cohort, although these factors may be associated with risk of OMJ.

In conclusion, our data suggest that oral BP use is strong risk factor for OMJ, and that age and sex-chemotherapy are also systemic risk factors in osteoporosis patients. The risk indices are moderately high, and allow the prediction of OMJ incidence.

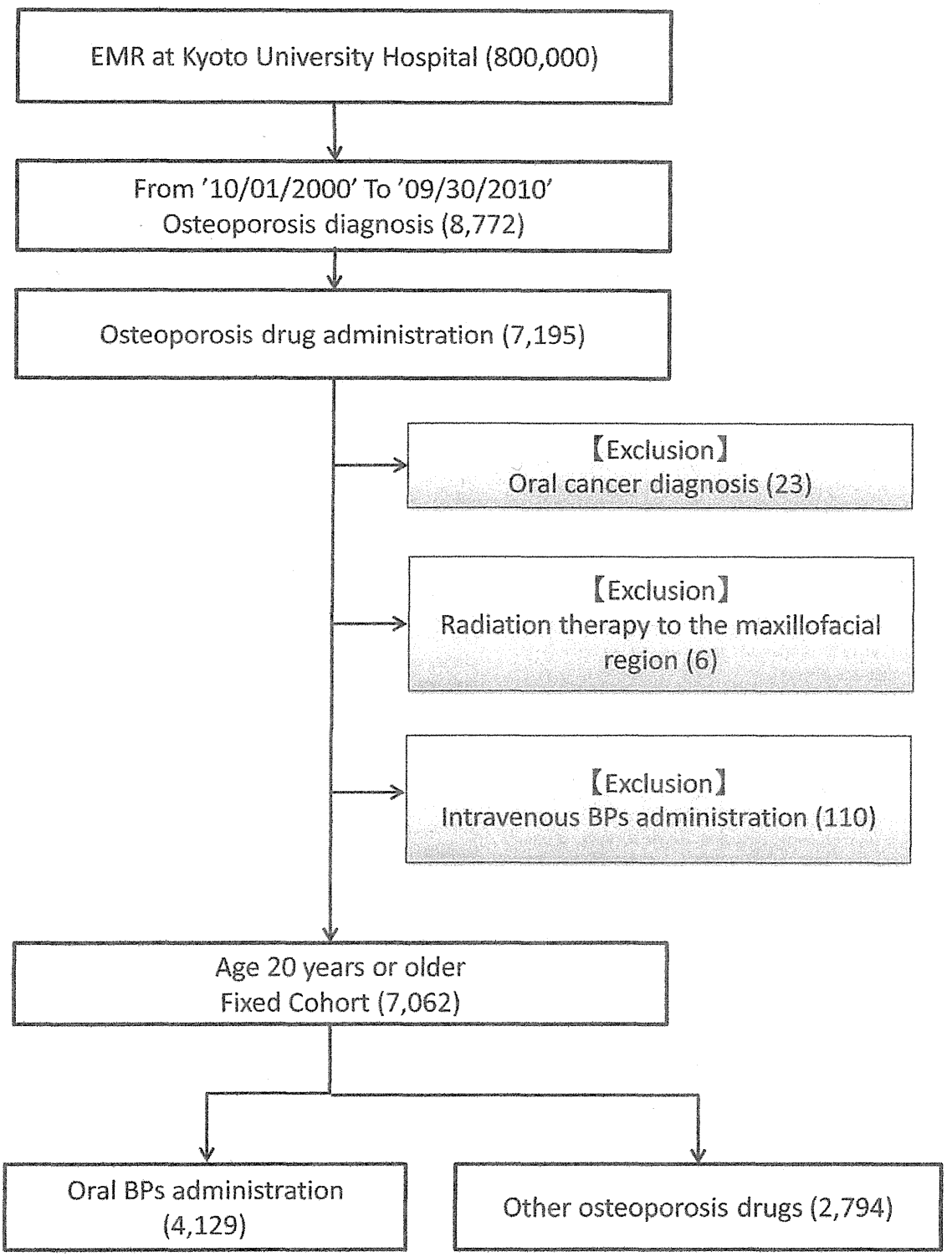

Figure 1. Patient flowchart. BPs = Bisphosphonates; EMR = Electronic Medical Records.
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Table 1. Characteristics of all patients taking any medications for osteoporosis and the subset of oral bisphosphonate users.

	Osteoporosis patients (n = 6,923)		Bisphosphonate users (n = 4,129)	
Median age (range)	65.0	(20-99)	65.0	(20-99)
Male, *n* (%)	1,539	(22.2)	814	(19.7)
Diabetes, *n* (%)	1,149	(16.6)	707	(17.1)
Steroid use, *n* (%)	4,442	(64.1)	2,934	(71.0)
Chemotherapy use, *n* (%)	807	(11.6)	551	(13.3)
Antirheumatic drugs use, *n* (%)	1,265	(18.2)	977	(23.6)
Biologic agents use, *n* (%)	186	(2.6)	145	(3.5)
Oral BPs administration*				
Etidronate, *n* (%)	N.A.		548	(13.2)
Alendronate, *n* (%)	N.A.		2,871	(69.5)
Risedronate, *n* (%)	N.A.		1,604	(38.8)
Minodronate, *n* (%)	N.A.		38	(0.92)

BPs = Bisphosphonates; N.A. = Not applicable.

*. In some cases, several oral BPs were prescribed for one patient.

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Table 2. Potential risk factors of osteomyelitis of the jaw in all osteoporosis patients and oral bisphosphonate users.

	Osteoporosis patients (46 cases, 6,923 patients)					Bisphosphonate users (41 cases, 4,129 patients)				
	Regression coefficient	Odds ratio	95% confidence interval		p	Regression coefficient	Odds ratio	95% confidence interval		p
Oral bisphosphonates, use vs. non-use	1.605	4.98	1.94	12.75	<0.01	-				
Age, +10 years	0.247	1.28	1.03	1.60	0.03	0.269	1.31	1.03	1.67	0.03
Sex, men vs. women	-0.911	0.40	0.14	1.14	0.09	-1.493	0.22	0.05	0.95	0.04
Diabetes, yes vs. no	0.574	1.78	0.86	3.68	0.12	-0.050	0.95	0.41	2.18	0.91
Steroid, use vs. non-use	-0.209	0.81	0.36	1.84	0.62	0.509	1.66	0.78	3.56	0.19
Chemotherapy, use vs. non-use	-0.925	0.40	0.09	1.66	0.21	-0.890	0.41	0.10	1.73	0.22
Antirheumatic drugs, use vs. non-use	-0.045	0.96	0.44	2.08	0.91	-0.294	0.75	0.31	1.77	0.51
Biologic agents, use vs. non-use	0.756	2.13	0.57	7.91	0.26	1.007	2.74	0.71	10.60	0.14
Sex-chemotherapy interaction	2.460	11.70	1.46	93.64	0.02	3.067	21.48	2.14	215.21	0.01

*Risk indices were calculated by the sum of risk factors of an individual patient multiplied by the regression coefficient.

doi: 10.1371/journal.pone.0079376.t002

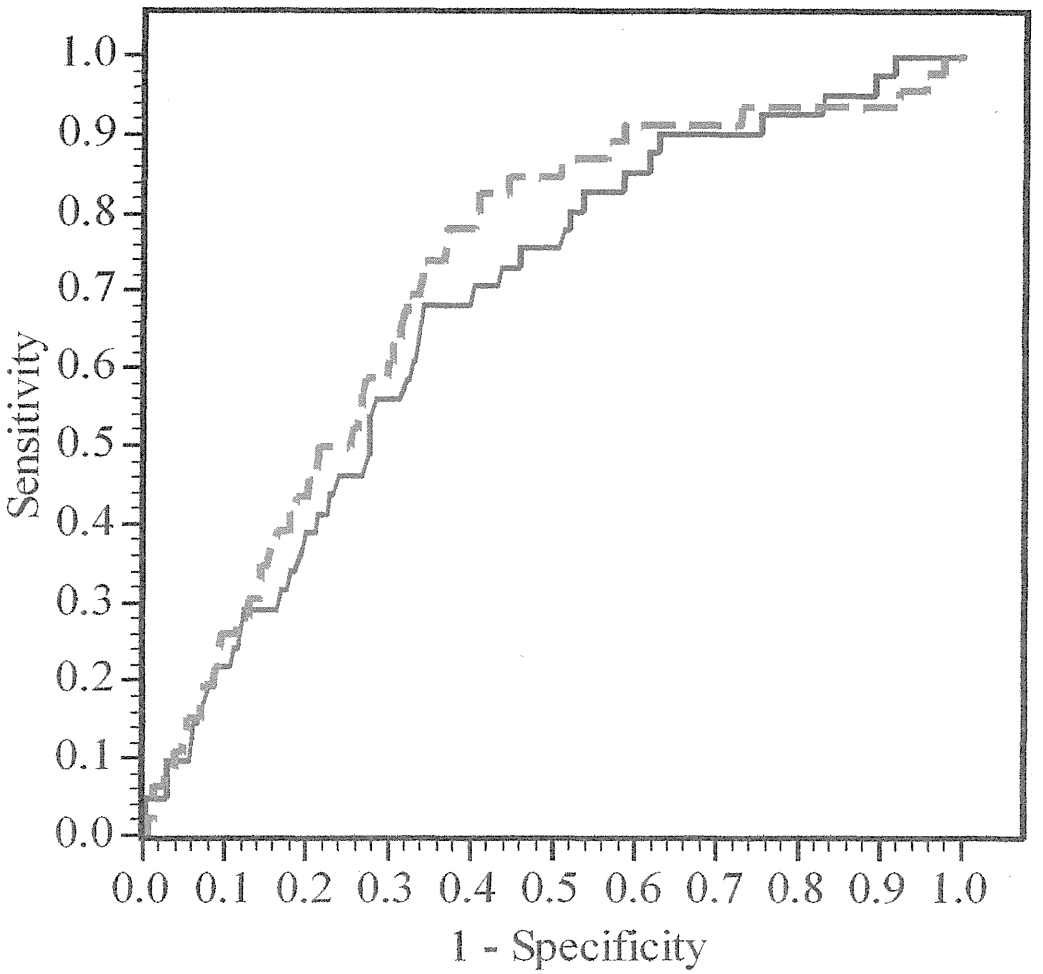

Figure 2. Receiver operating characteristic curves of risk indices in predicting the incidence of OMJ. Areas under the curve are 0.683 (95% confidence interval, 0.607 to 0.760) for the solid curve among oral BPs users and 0.718 (0.648 to 0.789) for the dashed curve in all patients.

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Supporting Information

Appendix S1. Diagnoses and 10th International Classification of Diseases codes for osteoporosis.
(DOCX)

Appendix S2. Drug and generic names for osteoporosis medications approved in Japan between November 2000 and October 2010.
(DOCX)

Appendix S3. Diagnoses and 10th International Classification of Diseases codes for case definition for osteomyelitis or osteonecrosis of the Jaw (version 2007, updated in January 2010).
(DOCX)

Author Contributions

Conceived and designed the experiments: TY MY ST KY ES MNS KA KT TN KB. Performed the experiments: TY KA. Analyzed the data: ST. Contributed reagents/materials/analysis tools: KY. Wrote the manuscript: TY. Contributed to the initial revision of the manuscript: MY ST KY ES. Contributed to the critical revision of the manuscript: KT TN KB. Agree with manuscript results and conclusions: TY MY ST KY ES MNS KA KT TN KB.

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Recent Clinical Evidence in Bisphosphonate-related Osteomyelitis of the Jaw: Focus on Risk, Prevention and Treatment

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Abstract: Bisphosphonates (BPs) are widely used for the treatment of a range of conditions involving bone, such as osteoporosis and bone metastases of cancer, and their efficacy has been confirmed. Nevertheless, a first case of bisphosphonate-related osteonecrosis of the jaw (BRONJ) as an adverse effect of BP treatment was reported in 2003, and several clinical studies since then have elaborated the risk, prevention and treatment of BRONJ or bisphosphonate-related osteomyelitis of the jaw (BROMJ). However, effective decision making on BP risk is hampered by a lack of accurate information for patients, physicians or dentists. Furthermore, the narrow definition of BRONJ used to date has precluded the wider development of clinical research on risk.

In this review, we discuss current issues in BROMJ, with a focus on risk, prevention and treatment. In particular, we reconsider the definition of BRONJ from the standpoint of clinical evidence. Finally, we propose a new strategy for the treatment of BROMJ.

Keywords: Absolute risk, bisphosphonate, osteonecrosis of the jaw, prevention, prognosis, relative risk, risk factors, treatment.

INTRODUCTION

Bisphosphonate (BPs) are widely used for the prevention and treatment of a range of bone conditions, including postmenopausal osteoporosis, Paget disease, hypercalcemia in malignancies, and osteolytic bone metastases of cancer or multiple myeloma [1, 2]. BPs can be administered orally and intravenously in a wide range of doses, dosing intervals, and duration of administration [1]. The biological action of BPs is to suppress farnesyl pyrophosphate synthase in the mevalonate biosynthetic pathway and inhibit the resorption of bone *via* the inactivation of osteoclasts [3]. Although this action accounts for the preventive or therapeutic efficacy of these agents, it also accounts for their uncommon skeletal-related events (SRE) or adverse effects [1, 2]. In particular, cases of osteonecrosis of the jaw (ONJ) have been reported as possible adverse effect of BPs since 2003 [4]. This condition is presently defined by the presence of exposed bone in the maxillofacial region for six to eight weeks [5-10], and patients with ONJ often encounter difficulties in sustaining their quality of life (QOL) [11].

Due to a lack of information, BP-related ONJ (BRONJ) was initially considered as difficult to treat, similarly to osteoradionecrosis of the jaw, and to be a largely different condition to osteomyelitis of the jaw (OMJ) [4, 12, 13]. Regrettably, however, a number of organizations, national regulatory agencies, medical specialty societies and clinicians disseminated information on the risk of BPs for ONJ without accurate data on incidence, risk factors, prognosis, or treatment [14], and thereby confounded both patients treated with BPs and medical and dental professionals. Many relevant studies and reviews have since appeared, however, and these early problems have been progressively resolved.

In this paper, we review clinical studies of BRONJ or BP-related OMJ (BROMJ) over the last 10 years, outline the problems identified, and then discuss current issues in BRONJ and BROMJ, with a focus on risk, prevention and treatment. In particular, we discuss the definition of BRONJ from the standpoint of clinical evidence. Finally, we propose a new strategy for the treatment of BROMJ.

METHODS

Literature Search Strategy and Research Questions

A systematic search of the English literature was conducted. The MEDLINE/PUBMED and Scopus databases were searched from January 1, 2003 to December 31, 2012. In the MEDLINE search, we entered the following Medical Subject Headings (MeSH): "diphosphonates"[MeSH Terms] OR "diphosphonates"[All Fields] OR "bisphosphonate"[All Fields] OR "bisphosphonate-associated osteonecrosis of the jaw"[MeSH Terms] OR "bisphosphonate-associated"[All Fields] AND ("jaw"[MeSH Terms] OR "jaw"[All Fields]) AND (hasabstract[text] AND "humans"[MeSH Terms] AND English[lang])). We additionally searched studies published from January 1, 2012 to December 31, 2012 in the Scopus database by using the following key words: (TITLE-ABS-KEY-AUTH("osteonecrosis of the jaw" OR "osteomyelitis of the jaw" OR "inflammation of the jaw") AND (LIMIT-TO (PUBYEAR, 2012)) AND (LIMIT-TO (LANGUAGE, "English")).

Titles and abstracts were reviewed to determine relevance. The search for clinical evidence included randomized

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controlled trials (RCT), cohort studies, case-control studies, cross-sectional studies, case series and literature reviews. Letters, animal studies and basic studies were excluded.

Our research questions in the review were as follows:

1. How much of the absolute risk of BROMJ is estimated to be accounted for by users of intravenous BPs?
2. How much of the absolute risk of BROMJ is estimated to be accounted for by users of oral BPs?
3. How much of the relative risk of OMJ is estimated by the incidence of OMJ in BPs users compared to non-users, regardless of BP type?
4. What are the risk factors of BROMJ?
5. Are there any prognosis markers for the incidence of BROMJ?
6. Are there any effective preventive measures for the incidence of BROMJ?
7. Are there any effective treatments for BROMJ?
8. Are there any new treatments for BROMJ?

In the review, we investigated the following information: year of electronic publication, country, setting, type of study, target population and number, main endpoint, diagnostician, type of BP, search procedure of BPs, risk index, risk ratio, risk factors, prognosis, and treatments. In addition, the evidence grade of studies was classified according to the 2010 American Heart Association guideline [15]. Here, we define meta-analyses as evidence 1a, RCTs as 1b, cohort studies as 2, and case-control studies as 3.

A unit of incidence rates was converted into the unit "per million person-years". All statistical analyses were performed using Stata 11.2 software (Stata Corporation, College Station, TX, USA).

RESULTS AND DISCUSSION

Definition of Epidemiological Terms

To aid understanding of this literature review, we first explain the epidemiological terms cumulative incidence, prevalence, incidence rate, absolute risk and relative risk, as follows.

Cumulative incidence refers to the number or proportion of a group (cohort) of people who experience the onset of a health-related event during a speci ed time interval [16]. In contrast, prevalence refers to the total number of individuals who have an attribute or disease at a particular time or particular period divided by the population at risk of having the attribute or disease at that time or midway through the period, respectively [16]. Although the term "prevalence" is thus inherently different from "cumulative incidence" in meaning, we include "prevalence" in "cumulative incidence" here because of the severely limited number of cross-sectional studies identified in the literature review. In contrast, incidence rate refers to the rate at which a new event occurs in a population, and is quite different from "cumulative incidence". Accordingly, we distinguish the term "incidence rate" from "cumulative incidence" in the review [16].

These risks are then grouped as "absolute risk", which means the number of events in a group divided by the total number of subjects in that group [16]. Moreover, we use the term "relative risk" to evaluate the risk of BPs for OMJ. This means the ratio of the risk of an event among the exposed to that among the unexposed [16].

History and Definitions of BROMJ

In 2003, Marx first suggested a possible association between the use of intravenous BPs and avascular necrosis of the jaw [4], and described 36 patients receiving pamidronate or zoledronate who had exposure of necrotic bone in the oral cavity. Since this sensational report, hundreds of cases of BRONJ cases have been reported [17-30] and a number of clinical studies published between 2003 and 2006 demonstrated the absolute risk or risk factors of BRONJ among patients using intravenous BPs [31-38]. In the same period, the manufacturers or the US Food and Drug Administration indicated the presence of a safety concern regarding the use of BPs [14]. Furthermore, some expert panels recommended the prevention and treatment of BP-associated ONJ notwithstanding that evidence for the association was limited, particularly among users of oral BPs [19, 20, 39-41]. Finally, in 2007, a position paper by the American Association of Oral and Maxillofacial Surgeons (AAOMS) proposed the establishment of BRONJ as a new disease entity with the following three characteristics: 1) current or previous treatment with a bisphosphonate; 2) exposed, necrotic bone in the maxillofacial region that has persisted for more than 8 weeks; and 3) no history of radiation therapy to the jaw [42]. Following this position paper, several associations stated definitions of BRONJ, BP-associated ONJ, or BP-ONJ which, despite the differences in naming, were commonly defined by the presence of exposed bone in the maxillofacial region [5-10, 43].

Here, we propose grouping cases of OMJ together with ONJ, because we consider it difficult to distinguish ONJ from OMJ, for two reasons: first, radiographic ndings in infected jaw bone in patients treated with BPs are similar to those in BP-induced ONJ even if necrotic bone cannot be clinically visualized [44-46]; and second, the presence of osteonecrosis is a common histopathologic nding in both BP-induced ONJ and OMJ [47]. These findings suggest that the condition of bone exposure in the oral cavity is not always caused by avascular necrosis of the jaw. Several studies or reviews have also regarded ONJ as the same as OMJ [48-51]. We therefore need to reconsider the definition of "BRONJ" according to this recent clinical evidence and pathological findings of the condition; in particular, such early identification of OMJ without long-term exposure of necrotic bone may be relevant to treatment.

How Much of the Absolute Risk of BROMJ is Estimated to be Accounted for by Users of Intravenous BPs?

Accumulated evidence has clarified that the risk of BROMJ is higher in patients taking intravenous BPs than oral BPs [37, 52-54]. In addition, most patients receiving intravenous BPs were considered to have cancer [7] and be at higher risk for infectious disease than those taking oral BPs. We therefore discuss incidence by route of administration.

Table 1. Characteristics of studies of cumulative risk of bisphosphonate-related osteomyelitis of the jaw among patients taking intravenous bisphosphonates.

Published Year	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
No. of studies	0	0	2	4	14	18	14	9	17	13
Country	USA	Italy	Greece	International		Germany	Canada	Australia	Japan	Others
No. of studies	24	18	8	7		6	4	4	3	19
Setting	Hospital	Multi-center		Single-center*		Health insurance data		Population-based		Others
No. of studies	55	20		6		6		2		2
Type of study	Meta-analysis		RCT	Cohort	Cohort w/o control		Case control		Cross-sectional	
No. of studies	1		21	5	59		2		3	
Target population	BC	MM	PC	LC	Cancer complex		Osteoporosis or Paget disease			Others
No. of studies	18	15	8	2	37		5			6
No. of population	<100		100-500		500-1000		1000-5000		5000>	
No. of studies	28		28		10		15		10	
Main endpoint	BROMJ			SRE including BROMJ			Codes of surgery or inflammation of the jaw			
No. of studies	47			39			5			
Diagnostician	Surgeons or oncologists			Dentists	Physicians	Investigators or committee			Others	Unclear
No. of studies	35			17	3	14			2	20
Search procedure	From pharmacy or prescription records				From medical or dental records			Marketing information		
No. of studies	79				9			3		
Evidence grade	Ia (meta-analysis)			Ib (RCT)			2 or 3 (controlled study)		4 (quasi-experimental study)	
No. of studies	1			21			7		62	
publication lists	98			61, 69, 72, 80, 93, 96, 101, 104, 105, 108, 111-113, 115, 116, 119, 120, 122, 123, 134, 135			37, 49, 53, 54, 106, 118, 133		10, 31, 32, 34-36, 55-60, 62-71, 73-79, 81-92, 94, 95, 97, 99, 100, 102, 103, 107, 109, 110, 114, 117, 121, 124-132	

BC = breast cancer; BPs = Bisphosphonate; BROMJ = bisphosphonate-related osteomyelitis of the jaw; LC = lung cancer; MM = multiple myeloma; PC = prostate cancer; RCT = randomized clinical trial; SRE = skeletal-related events; w/o = without.
* Studies were conducted in a single center, excluded hospitals.

(Table 1) show characteristics of the literature concerning cumulative risk of BROMJ among patients taking intravenous BPs. A total of 91 papers describing the cumulative risk of BROMJ were identified [10, 31, 32, 34-37, 49, 53-135], the largest number of which came from the US, followed by Italy, Greece and other countries. Most studies were conducted in hospitals, and were aimed at investigating the cumulative incidence or risk factors of BROMJ. More than half of the 91 studies were cohort studies, although almost none of these had a control group, in other words patients who were not treated with BPs. Further, 21 of the 91 studies were conducted as RCTs, but with efficacy of BPs or SREs as main outcome, and the incidence of BROMJ as a secondary endpoint only. The cumulative incidence of BROMJ in those studies ranged from 0% to 51.8%, or the incidence rate ranged from 0.70 per 100 patients to 5.5 per 1,000 person-years.

We summarized the characteristics of studies of BROMJ among patients taking intravenous BPs which had an evidence level of 3 or better (Tables **2** and **3**). The cumulative incidence of BROMJ in multicenter RCTs was extremely low, ranging from 0% to 3.5%, with a median incidence of 0.6% (Table **2**). In contrast, the cumulative incidence of BROMJ in controlled, observational studies ranged from 0.34% to 14.8%, with a median incidence of 5.0% (Table **3**). These findings appear to indicate a large difference between these studies in absolute risk.

We speculate that the difference in absolute risk between studies is partly due to differences among the investigators of BROMJ in the various studies. In particular, the diagnostic criteria for BROMJ or the background of those diagnosing BROMJ was unclear in prospective studies because BROMJ was just one SRE or secondary endpoint. This might have

Table 2. Incidence of bisphosphonate-related osteomyelitis of the jaw among patients taking intravenous bisphosphonates in studies with an evidence level of 1.

No.	Author	Setting	Target	Kind of BP	End Point*	Diagnosis	Outcomes/Users	Incidence (%)	Evidence
98	Mauri	N.A.	BC	P, ZA, I, C, R	0	unclear	13/3,987	0.2	1a
61	Lyles	MC	HF	ZA	1	physicians	0/1,054	0.0	1b
69	Grbric	MC	OSP	ZA	1	AC	1/3,875	0.03	1b
72	Brufsky	MC	BC	ZA	1	unclear	0/1,652	0.0	1b
80	Musto	MC	MM	ZA	1	unclear	1/81	1.2	1b
93	Hines	SC	BC	ZA	1	OMS	1//274	0.4	1b
96	Brufsky	MC	BC	ZA	1	AC of ONJ	0/301	0.0	1b
101	Guarneri	MC	BC	P, ZA, C with BV	1	unclear	2/233-10/425	0.9-2.4	1b
104	Gimsing	MC	MM	P	1	questionnaire	30mg: 2/252, 90mg: 8/250	30mg: 0.8, 90mg: 3.2	1b
105	Stopeck	MC	BC	ZA, DMAB	1	AC of ONJ	ZA: 14/1,013, DMAB: 20/1,026	ZA: 1.4, DMAB: 2.0	1b
108	Morgan	MC	MM	ZA, C	1	dentists	ZA: 35/983, C: 3/979	ZA: 3.5, C: 0.3	1b
111	Henry	MC	CA, MM	ZA, DMAB	1	AC of ONJ	ZA: 11/878, DMAB: 10/878	ZA: 1.3, DMAB: 1.1	1b
112	Fizazi	MC	PC	ZA, DMAB	1	AC	ZA: 12/945, DMAB: 22/943	ZA: 1.2, DMAB: 2.3	1b
113	Coleman	MC	BC	ZA	1	investigators	11/1,590	0.7	1b
115	Gnant	MC	BC	ZA	1	investigators or patients level	0/900	0.0	1b
116	Pivot	SC	BC	I	1	investigators or patients level	2/334	0.6	1b
119	Saad	MC	CA, MM	ZA, DMAB	1	AC of dental experts	ZA: 37/2,836, DMAB: 52/2,841	ZA: 1.3, DMAB: 1.8	1b
120	Brufsky	MC	BC	ZA	1	investigators and AC of ONJ	0/602	0.0	1b
122	Coleman	MC	BC	ZA	1	investigators	17/1,686	1.1	1b
123	Safra	SC	BC	ZA	1	investigators	0/47	0.0	1b
134	Scagliotti	MC	LC	ZA, DMAB	1	unclear	ZA: 3/406, DMAB: 3/395	ZA: 0.7, DMAB: 0.8	1b
135	Scagliotti	MC	LC	ZA	1	investigators	1/226	0.4	1b

AC = adjudication committee; BC = breast cancer; BPs = bisphosphonates; BV = bevacizumab; C = clodronate; CA = cancer patients; DMAB = denosumab; HF = hip fracture patients; I = ibandronate; LC = lung cancer; MC = multicenter; MM = multiple myeloma; N.A. = not applicable; OMS = oral and maxillofacial surgeons; ONJ = osteonecrosis of the jaw; OSP = osteoporosis; P = pamidronate; PC = prostate cancer; R = risedronate; SC = single center; ZA = zoledronic acid.
* Endpoint 0 means ONJ and 1 means skeletal related events including ONJ.

resulted in underestimation of the incidence of BROMJ. In addition, we suspect that differences in the settings or target populations of the studies mainly influenced absolute risk; in other words, participants in the clinical trials may have been generally healthier than the subjects of the clinical observational studies. On the other hand, the subjects in clinical observational studies may have had several primary illnesses and required substantial medical treatment, including BPs. Moreover, differences between these studies may have resulted from differences in the duration of exposure to BPs, although we were unable to investigate duration in detail. To sum up, any interpretation of our results should be done with due regard to study design, setting, target population, sample size, definition of outcome, and diagnostician.

Table 3. Incidence of bisphosphonate-related osteomyelitis of the jaw among patients taking intravenous bisphosphonates in studies with an evidence level of 2 or 3.

No.	Author	Setting	Target	Kind of BP	End Point*	Diagnosis	Outcomes/ BPs users	Incidence (% or Rate)	Evidence
37	Zavras	HIP	CA, MM	P, ZA	2	ICD-9 code	20/5,850	0.34	2
53	Tennis	HIP	CA	I, P, ZA	0	ICD-9 or CPT and chart review	15/2,876	5.3 per 1,000 person-years	2
54	Yamazaki	HOSP	CA	INC, P, ZA	0	OMS	4/27	14.8†	2
106	Skrepnek	HIP	CA, OSP	P, ZA	2	ICD-9 code	CA: 12/6,276, OSP: 21/2,321	CA: 0.43 OSP: 0.90	2
133	Beuselinck	HOSP	RCC	ZA	1	patient level	5/49	9.6	2
49	Wilkinson	HIP	CA	P, ZA	2	ICD-9 code	95/14,349	5.5 per 100 patients	3
118	Baillargeon	HIP	OSP	E, I, P, ZA	2	ICD-9 code	9/2,296	0.70 per 100 patients	3

BPs = bisphosphonates; CA = cancer patients; CPT = current procedural terminology; E = etidronate; HIP = health insurance plan data; HOSP = hospital; I = ibandronate; INC = incadronate; ICD = international classification of diseases; MM = multiple myeloma; OMS = oral and maxillofacial surgeons; OSP = osteoporosis; P = pamidronate; RCC = renal cell carcinoma patients; ZA = zoledronic acid.

* Endpoint 0 means osteonecrosis of the jaw (ONJ), 1 means skeletal related events including ONJ, and 2 means jaw surgery or inflammation of the jaw code.

† Cumulative incidence of bisphosphonate-related osteonecrosis of the jaw after tooth extraction.

Denosumab is a human monoclonal antibody against receptor activator of nuclear factor kappa-B ligand. Several studies have shown the superiority of this agent to BPs in the treatment of bone metastases and prevention of SRE in cancer patients [105, 111, 112, 119, 134]. Notably, these studies have also indicated that denosumab has a similar risk for OMJ as BPs. The absolute risk of OMJ was estimated to range from 0.8% to 2.3%. Given that evidence for the risk of OMJ with denosumab remains limited, however, particular vigilance against the possibility of adverse effects in these patients is required.

How Much of the Absolute Risk of BROMJ is Estimated to be Accounted for by Users of Oral BPs?

The cumulative incidence of BROMJ in patients taking oral BPs ranged from 0% to 7.8% [10, 37, 51, 54, 62, 106, 136-150], or the incidence rate ranged from 6.3 to 366 per million person-years [53, 146, 151, 152]. We abstracted those studies with an evidence level of 3 or better (Table 4), among which cumulative incidence was estimated to range from 0% to 4.3%, or the incidence rate from 6.3 to 366 per million person-years.

Due to the low occurrence of BROMJ in patients treated with oral BPs, initial studies estimated incidence by anticipating the total number of individuals who had been prescribed oral BPs [62, 144]. More recently, however, population-based or larger hospital-based studies, as well as administrative data have allowed an understanding of the absolute risk of BROMJ in patients taking oral BPs [51, 53, 106, 146, 151, 152]. From these studies and our previous study, the cumulative risk of OMJ with oral BPs is less than 1% in patients with osteoporosis, and the incidence risk is considered to be low.

How Much of the Relative Risk of OMJ is Estimated by the Incidence of OMJ in BPs Users Compared to Non-users, Regardless of BP Type?

A meta-analysis which extracted data from 15 RCTs (n = 10,694) showed that treatment with zoledronic acid was signi cantly associated with the occurrence of ONJ (M-H pooled odds ratios (OR) = 3.2, 95% confidence interval (CI), 1.7–8) compared with no use [98]. In contrast, a meta-analysis of other data extracted from three RCTs (n = 736) showed no significant association between intravenous BPs and ONJ (pooled relative risks (RR) = 4.0, 95% CI, 0.44-35.8) [2]. Six observational studies reported the relative risk of BROMJ in patients treated with intravenous BPs while 10 observational studies reported the risk in patients treated with oral BPs (Table 5). The estimated OR, RR or hazard risks in patients treated with intravenous BPs ranged from 1.6 (95% CI, 0.71-3.8) to 299.5 (95% CI, 70-1282). Of these studies, only one found no significant association between intravenous BPs and OMJ [118], whereas the rest showed an increased risk of OMJ with intravenous BPs, with significance [37, 49, 52-54, 153]. Similarly, four studies found no significant or inverse association between oral BPs and OMJ [37, 53, 54, 154], whereas the rest showed an increased risk of OMJ with oral BPs, ranging from 2.2 (95% CI, 1.2-4.3) to 15.5 (95% CI, 6.0-38.7) [50, 51, 146, 151-153]. Overall, both intravenous and oral BPs may increase the risk of OMJ, although these studies slightly differed in endpoint characteristics (*e.g.* OMJ or Jaw surgery code), sample size, target population and number, and presence of adjustment for confounding. A conclusive answer awaits additional meta-analysis or larger clinical observational studies.

What are the Risk Factors of BROMJ?

More than one hundred studies have examined risk factors associated with BROMJ or prognosis. In particular,