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Mineral content of calcium and magnesium in the serum and longitudinal periodontal progression in Japanese elderly smokers

Akihiro Yoshihara, Masanori Iwasaki and Hideo Miyazaki

Department of Oral Health Science, Division of Preventive Dentistry, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan

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Abstract

Objective: Evidence from physiological and clinical studies regarding the mechanism by which calcium and magnesium are associated with periodontal disease, adjusted for smoking habits, is lacking. This longitudinal study evaluated the association of serum calcium levels and the calcium/magnesium (Ca/Mg) ratio with periodontal disease progression among smokers and non-smokers.

Material and Methods: A total of 309 subjects aged 73 years were included. Follow-up surveys were carried out every year for 6 years. After dividing subjects into smokers and non-smokers, multiple logistic regression analysis for men was performed to evaluate the relationship between the serum Ca/Mg ratio and periodontal disease progression. We used the 25th percentile of periodontal disease events for 6 years as the dependent variable and seven other variables, including quartiles for serum Ca/Mg ratios, as independent variables.

Results: There was a clear dose–response relationship of Ca/Mg ratio quartiles for periodontal disease events among smokers. Significant differences in odds ratios in the first and second quartiles were seen compared with the fourth quartile (reference): 6.28 ($p = 0.014$) and 5.96 ($p = 0.022$), respectively. However, there was no significant dose–response relationship among non-smokers.

Conclusion: A low serum Ca/Mg ratio was significantly associated with periodontal disease progression in Japanese elderly smokers.

Key words: calcium; elderly; epidemiology; magnesium; periodontal disease; smoking habits

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Calcium and magnesium are the most abundant minerals in humans. About 99% of the total calcium in the human body exists in the bones and teeth, providing a structural function; the remaining 1% is found in tissues and fluids and is crucial for the maintenance of cell metabolism, nerve transmission, and muscle contraction (Edwards 2005). In addition, approximately 50–60% of magnesium is in the skeleton, and subjects with low bone mass tend to have chronic magnesium deficits. Low dietary magnesium levels are a risk factor for osteoporosis (Rude et al. 2009, Nielsen 2010).

Conflict of interest and source of funding statement

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Calcium plays many important roles in the synthesis, release, and receptor responsiveness to neurotransmitters (Robinson et al. 2010). Magnesium acts as a critical cofactor for hundreds of enzymes and a direct antagonist of intra-cellular calcium (Paolisso & Barbagallo 1997, Barbagallo et al. 2003) and also influences catecholaminergic, serotonergic, and cholinergic transmission (Banki et al. 1985). On the other hand, the roles of calcium and magnesium in the process of calcium absorption in the gastrointestinal tract are synergistic, as the production of active vitamin 1,25$(OH)_2D_3$, indispensable in calcium absorption from food, is a magnesium-dependent process, and serum content is similar to that of calcium and magnesium (Unkiewicz-Winiarczyk et al. 2009).

Researchers have been exploring the role played by minerals in the aetiology and/or progression of periodontal disease for more than four decades. Recent studies have shown a significant relationship between dietary calcium, serum calcium, and periodontal disease (Nishida et al. 2000, Krall et al. 2001, Amarasena et al. 2008) as well as between the serum magnesium/calcium (Mg/Ca) ratio and periodontal disease (Meisel et al. 2005).

Confounding and effect modification are of increasing importance as periodontal research addresses putative associations between periodontal disease and systemic disease. This is especially pertinent when dealing with smoking, as smoking is a major risk factor for both periodontal disease and several systemic diseases (Hyman 2006). However, evidence from physiological and clinical studies regarding the mechanism by which calcium and magnesium are associated with periodontal disease, adjusted for smoking habits, is lacking. The purpose of the present study was to evaluate the independent association of serum calcium and the Ca/Mg ratio with longitudinal periodontal disease progression in Japanese elderly subjects, taking smoking habits into consideration.

Material and Methods

Subjects

In 1998, a longitudinal interdisciplinary study of ageing was initiated to evaluate the relationship between oral health and general health status. Initially, questionnaires were sent to all 4542 residents aged 70 years (born in 1927) in Niigata City, Japan. Among them, after dividing by gender, 600 people were randomly selected to have approximately the same number of each gender for the baseline survey. Participants agreed to undergo medical and dental examinations and signed informed consent forms regarding the protocol, which was approved by the Ethics Committee of Niigata University School of Dentistry. The study was carried out according to the rules of the Helsinki Declaration.

A total of 432 subjects who turned 70 in 1998 and were 73 years of age in 2001 had annual dental examinations. Follow-up surveys have been carried out every year in June over 6 years (2001–2007), that is, seven times in 6 years, using the same methods as those used at baseline. All subjects were Japanese and did not require special care for their daily activities. Subjects were homogenous in terms of race, and we restricted the age to 73 years at baseline to exclude the influence of age on results.

Periodontal conditions were estimated for subjects with at least one remaining tooth. The periodontal examination included the assessment of clinical attachment level (CAL) at six sites around each tooth. Probing was performed using a pressure constant probe (Vivacare TPS Probe®, Schaan, Liechtenstein) at a probing force of 20 g and rounded to the nearest whole millimetre. The periodontal examination was carried out by four trained dentists under sufficient illumination using artificial light. Calibration of the examiners was carried out in volunteer patients at the Faculty Hospital. As determined by replicate examinations in 10 patients, the percent agreement ($\pm$ 1 mm) ranged from 83.3% to 100% for CAL. The κ ranged from 0.74 to 1.00 for CAL.

We conducted personal interviews with subjects to obtain information regarding the exposed years to smoking and the use of inter-dental brushes or dental floss. Blood samples at baseline were taken on the day of the dental examination, and calcium and magnesium levels were determined using the OCPC method and the Xylidyl blue method, respectively. All laboratory tests were conducted at a commercial laboratory (BML Inc., Tokyo, Japan).

We measured bone stiffness to evaluate bone mineral density (BMD) using an Achilles ultrasound bone densitometer (Lunar Corporation, Madison, WI, USA). The ultrasound densitometer simultaneously measures speed of sound (SOS) in m/second and broadband ultrasound attenuation (BUA) in dB/MHz. These measurements are highly correlated with BMD of the os calcis (Lunar Corporation 1991). Based on this association, stiffness results are presented for the os calcis. The Achilles provides a stiffness index by combining BUA and SOS (stiffness = [0.67 $\times$ BUA]+[0.28 $\times$ SOS]–420). The stiffness is expressed as the percent deviation from expected young normal 20-year-old subjects.

Statistical analysis

The smoking status, serum calcium, and Ca/Mg ratio were determined at baseline. A total of 309 subjects who participated in all follow-up surveys over 6 years were analysed. The differences in serum calcium, magnesium, Ca/Mg ratio, periodontal condition, and BMD were compared between smokers and non-smokers. The Mann–Whitney *U*-test, *t*-test, and χ^2 test were used when applicable. In the longitudinal study, a change in the loss of attachment of 3 mm or greater in 1 year at any site was counted as a periodontal disease event (Brown et al. 1994). Teeth with one disease event were excluded from additional-year assessments because if we did not exclude teeth after they had one event, we had the possibility of counting a double for a tooth. In this case, the findings might be influenced by the local condition of each tooth. Finally, the number of teeth with events over 6 years per person was calculated. After subjects were divided into smokers and non-smokers, we categorized subjects into quartiles according to the serum calcium level and Ca/Mg ratio. Periodontal disease events over 6 years for each quartile were calculated, and the tendencies of the events for each serum item were evaluated by the test for trends across ordered groups. Furthermore, we carried out Poisson regression analysis to determine the relationship between periodontal disease progression and serum calcium level and the Ca/Mg ratio because the number of periodontal disease event was skewed to lower values. In this model, the number of teeth with periodontal disease events during 6 years was used as the dependent variable and each quartile of both serum calcium and Ca/Mg ratio was used as an independent variable. After we divided the subjects into men and women, multiple logistic regression analysis was performed to evaluate the relationship between serum Ca/Mg ratio and periodontal disease events during 6 years. We selected the 25th percentile of periodontal disease events as the dependent variable (0: $<$25th percentile, 1: $\geqslant$25th percentile). The participants were classified into quartile groups according to their number of periodontal events and then divided into two groups (highest quartile *versus* the other three groups combined). This is because we suspected a specific amount of inflammatory burden was required to cause a decline in the serum Ca/Mg ratio more clearly. In addition, we selected serum Ca/Mg ratio (quartiles), bone stiffness, number of teeth present at baseline, mean CAL at baseline, and use of inter-dental brushes or dental floss (yes, no) at baseline as independent variables. The multiple logistic regression analysis was carried out only for a man because even though there were some smokers among women ($n = 11$), all the smokers belonged to the group with <25th percentile of periodontal disease events. Finally, after dividing subjects into tertiles according to the exposed years to smoking, we compared the odds ratios of 25th percentile of periodontal disease events during 6 years (1: <25th percentile, 0: ⩾25th percentile) with the serum Ca/Mg ratio adjusted for gender, the number of remaining teeth at baseline, mean CAL at baseline, bone stiffness, and use of inter-dental brushes or dental floss.

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

Table 1. Comparison of selected baseline characteristics between smokers and non-smokers

Variables	Smokers ($n = 151$)	Non-smokers ($n = 158$)	p-value
Calcium (mEq/l)	4.52 ± 0.19	4.58 ± 0.23	0.012*
Magnesium (mg/dl)	2.12 ± 0.15	2.13 ± 0.16	0.556*
Gender (male/female)	140/11	26/132	<0.0001†
Number of present teeth	17.1 ± 9.6	17.5 ± 9.3	0.737‡
Mean clinical attachment level (mm)	3.6 ± 1.1	3.1 ± 0.8	<0.001‡
≥ 4 mm of clinical attachment level (%)	46.5 ± 28.4	33.4 ± 26.0	0.0001‡
≥ 6 mm of clinical attachment level (%)	12.2 ± 16.6	4.2 ± 8.5	<0.0001‡
Bone stiffness (%)	70.2 ± 11.2	61.5 ± 10.3	<0.0001‡

*t-test.
†χ^2-test.
‡Mann–Whitney U-test.

Table 2. Mean values of periodontal disease events for each quartile of serum calcium and calcium/magnesium (Ca/Mg) ratio in smokers

	Mean (SD)	Periodontal disease events*				
		mean (SD)	95% CI	IRR†	95% CI	p-value
Calcium (mEq/l)						
First	4.35 (0.09)	7.7 (5.8)	6.2–9.2	1.00 (reference)	–	–
Second	4.50 (0.00)	7.0 (4.3)	5.4–8.6	0.90	0.77–1.06	0.217
Third	4.64 (0.05)	6.8 (4.8)	5.3–8.3	0.87	0.75–1.01	0.068
Fourth	4.89 (0.10)	4.1 (3.7)	2.5–6.3	0.57	0.45–0.73	<0.0001
p-value for trend = 0.051						
Ca/Mg ratio (mol/mol)						
First	3.20 (0.13)	8.4 (5.2)	6.7–10.0	1.00 (reference)	–	–
Second	3.44 (0.04)	7.3 (5.7)	5.5–9.1	0.87	0.75–1.02	0.090
Third	3.62 (0.07)	6.3 (4.8)	4.5–8.0	0.75	0.63–0.90	0.001
Fourth	3.97 (0.19)	5.5 (4.1)	4.1–6.9	0.66	0.55–0.78	<0.0001
p-value for trend = 0.015						

*Number of teeth with periodontal disease progression during 6 years.
†Incidence rate ratios by Poisson regression.
95% CI, 95% confidence interval.

Results

The findings in this study were based on the Japanese elderly population. The mean CAL, serum calcium, and serum magnesium at baseline were 3.4 ± 1.0, 4.55 ± 0.21, and 2.12 ± 0.16 for the study subjects ($n = 309$) and 3.3 ± 1.1, 4.54 ± 0.23, and 2.13 ± 0.15 for the subjects who dropped out during the study ($n = 123$), respectively. The differences between these groups were not statistically significant.

Table 1 shows the differences in serum calcium levels, magnesium levels, oral status, and bone stiffness among smokers and non-smokers at baseline. The mean exposed years to smoking among smokers was 36.1 ± 15.0 years. The serum calcium level was significantly lower in smokers than in non-smokers. On the other hand, periodontal status such as mean CAL, percentage of teeth with ⩾4 mm CAL, percentage of teeth with ⩾6 mm CAL, and bone stiffness were significantly higher in smokers than in non-smokers.

Differences in the distribution of periodontal disease events during the 6 years by serum calcium level and Ca/Mg ratio among smokers are shown in Table 2. There was a significant negative tendency between Ca/Mg ratio and the mean value of periodontal disease events by the test for trend across ordered groups. The p-value for the trend was 0.015. According to the results of Poisson regression analysis, a high Ca/Mg ratio was significantly associated with fewer periodontal disease events. The incidence rate ratios of periodontal disease events the third and fourth Ca/Mg ratio quartiles compared with the first quartile were 0.75 ($p = 0.001$) and 0.66 ($p < 0.0001$), respectively. Differences in the distribution of periodontal disease events during 6 years according to serum calcium level and Ca/Mg ratio among non-smokers are shown in Table 3. There was no significant tendency between periodontal disease events and serum calcium level or Ca/Mg ratio.

Table 4 shows the findings of logistic regression analysis and associated p-values for the relationship between Ca/Mg ratio and 25th percentile of the number of teeth with periodontal disease progression during 6 years in men. There was a clear dose–response relationship of the Ca/Mg ratio quartiles for periodontal disease events among smokers. Significant differences in the odds ratios in the first and second quartiles were seen compared with the fourth quartile (reference): 6.28 ($p = 0.014$) and 5.96 ($p = 0.022$), respectively.

The odds ratios between the Ca/Mg ratio and the 25th percentile of periodontal disease events by tertiles of exposed years to smoking are shown in Fig. 1. The exposed years to smoking for the first, second, and third tertiles were 0 ± 0, 19.8 ± 9.5, and 45.4 ± 7.7 years, respectively. The odds ratios were 2.13 for the first tertile, 6.25 for the second tertile, and 33.33 ($p = 0.004$) for the third tertile. There was a clear dose–response relationship between the exposed years to smoking and the odds ratios.

Table 3. Mean values of periodontal disease events for each quartile of serum calcium and calcium/magnesium (Ca/Mg) ratio in non-smokers

	Mean (SD)	Periodontal disease events*				
		mean (SD)	95% CI	IRR†	95% CI	*p*-value
Calcium (mEq/l)						
First	4.30 (0.20)	5.4 (4.8)	3.8–7.1	1.00 (reference)	–	–
Second	4.50 (0.00)	5.7 (4.9)	4.2–7.3	1.05	0.87–1.28	0.607
Third	4.65 (0.05)	4.9 (4.3)	3.6–6.1	0.90	0.74–1.09	0.264
Fourth	4.86 (0.12)	4.8 (4.4)	3.3–6.3	0.88	0.71–1.08	0.220
p-value for trend = 0.438						
Ca/Mg ratio (mol/mol)						
First	3.19 (0.13)	4.7 (3.8)	3.4–6.0	1.00 (reference)	–	–
Second	3.44 (0.05)	5.6 (5.1)	3.9–7.2	1.19	0.97–1.46	0.087
Third	3.63 (0.07)	6.2 (5.2)	4.5–7.9	1.33	1.09–1.62	0.005
Fourth	3.94 (0.26)	4.4 (4.0)	3.2–5.6	0.95	0.72–1.16	0.608
p-value for trend = 0.798						

*Number of teeth with periodontal disease progression during 6 years.
†Incidence rate ratios by Poisson regression.
95% CI, 95% confidence interval.

Table 4. Logistic regression and associated *p*-values for the relation between calcium/magnesium (Ca/Mg) ratio and 25th percentile of the number of teeth with periodontal disease progression during 6 years in men

Independent variables	Dependent variable			
	periodontal disease events*			
	odds ratio	standard error	95% CI	*p*-value
Smokers				
Ca/Mg ratio first (dummy)	6.28	4.71	1.45–27.28	0.014
Ca/Mg ratio second (dummy)	5.96	4.63	1.30–27.34	0.022
Ca/Mg ratio third (dummy)	2.86	2.27	0.60–13.59	0.187
Ca/Mg ratio fourth (reference)	1.00	–	–	–
Stiffness	0.99	0.02	0.95–1.04	0.724
Number of present teeth	1.14	0.05	1.05–1.24	0.001
Mean CAL	1.76	0.45	1.07–2.91	0.026
Use of inter-dental brushes or dental floss (1: yes, 0: no)	1.27	0.57	0.53–3.08	0.593
Pseudo $R^2 = 0.155$, $p = 0.0013$				
Non-smokers				
Ca/Mg ratio first (dummy)	1.23	1.06	0.23–6.70	0.810
Ca/Mg ratio second (dummy)	1.36	1.32	0.20–9.12	0.752
Ca/Mg ratio third (dummy)	2.44	2.18	0.43–14.00	0.317
Ca/Mg ratio fourth (reference)	1.00	–	–	–
Stiffness	0.98	0.03	0.92–1.04	0.508
Number of present teeth	1.25	0.10	1.08–1.46	0.004
Mean CAL	5.19	3.51	1.38–19.53	0.015
Use of inter-dental brushes or dental floss (1: yes, 0: no)	1.25	0.82	0.34–4.55	0.739
Pseudo $R^2 = 0.235$, $p = 0.0092$				

*25th percentile of the number of teeth with periodontal disease progression during 6 years.
95% CI, 95% confidence interval; CAL, clinical attachment level.

Discussion

Although 123 subjects dropped out during the study, there were no significant differences in the mean CAL, serum calcium levels, and serum magnesium levels at baseline between the study subjects and the subjects who dropped out. We believe that subjects in this study are representative of the community at large.

In this longitudinal investigation, a significant association was found between the number of periodontal disease events over 6 years and the serum Ca/Mg ratio among Japanese elderly smokers. We observed a clear, inverse independent dose–response relationship between the Ca/Mg ratio and periodontal disease progression.

Smoking is known to be an effect modifier in periodontal disease (Hyman 2006). Because smoking was considered an effect modifier in our model, a significant relationship between Ca/Mg ratio and periodontal disease might only exist among smokers. In addition, compared with non-smokers, a lower content of calcium was observed in smokers. Previous studies showed that smoking was associated with impaired intestinal calcium absorption (Rapuri et al. 2000, Unkiewicz-Winiarczyk et al. 2009).

According to the results of the present study, smokers in the first and second quartiles of serum Ca/Mg ratio had significantly higher odds for periodontal disease events than those in the fourth quartile. Based on these results, we may infer that the serum Ca/Mg ratio, rather than the serum calcium level, is a more important factor involved in periodontal disease progression. However, our findings were partially contrary to the results of a previous study (Meisel et al. 2005) in which an increased serum Mg/Ca ratio was significantly associated with less attachment loss, although that study was cross-sectional and reported that periodontal parameters were inversely related to serum calcium concentrations. We could not confirm the reasons for our findings, and comparisons with the literature are difficult because of a lack of studies. In our study, we divided subjects into smokers and non-smokers because smoking affects periodontal disease and several systemic diseases (Hyman 2006). More studies, including prospective trials, are necessary to understand the exact nature of the relationship between periodontal disease and serum calcium and magnesium, taking smoking habits or relevant factors such as serum nicotine levels into consideration.

The interactive association between calcium and magnesium has been widely studied in various chronic diseases as well as BMD. One study showed that if serum calcium levels were lower than the mean values, BMD was affected more by serum magnesium than calcium levels (Song et al. 2007). An animal study showed that dietary magnesium reduction-induced bone loss was associated with decreased serum magnesium and elevated serum calcium levels (Rude

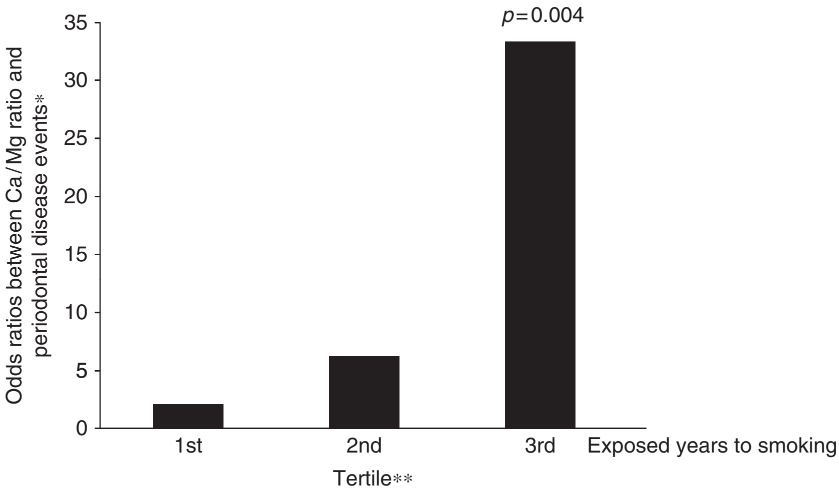

Fig. 1. Odds ratios between the calcium/magnesium (Ca/Mg) ratio and periodontal disease event by tertiles of exposed years to smoking adjusted for gender, the number of remaining teeth at baseline, mean clinical attachment level at baseline, stiffness at baseline, and use of inter-dental brushes or dental floss at baseline. *25th percentile of the number of teeth with periodontal disease progression during 6 years (1: $<$25th percentile, 0: $\geq$ 25th percentile). **First tertile, 0 ± 0 exposed years to smoking; second tertile, 19.8 ± 9.5 exposed years to smoking; third tertile, 45.4 ± 7.7 exposed years to smoking.

et al. 2004). These results suggest that calcium and magnesium have a cellular antagonism and that balancing calcium and magnesium is important to maintain optimal bone density (Kosch et al. 2001).

On the other hand, the serum Ca/Mg ratio was not associated clearly with periodontal disease events during 6 years among non-smokers. Smoking is known to be a risk factor for periodontal disease and decreases in BMD**.** We believe that this discrepancy can be partially explained by the fact that smoking alters systemic immune response and homeostasis.

Many previous reports indicated a significant association between smoking and periodontal disease progression. Nicotine is thought to be the major harmful substance in tobacco. Nicotine derivatives are also known to be vasoconstrictive not only on peripheral vessels but also on coronary, placental, and gingival blood vessels (Leow et al. 2006). Tobacco use may reduce the functional activities of polymorphonuclear leucocytes including chemotaxis and phagocytosis (Persson et al. 2001). Likewise, smoking also has significant systemic effects on plasma IgG levels (Fredriksson et al. 2002).

In conclusion, the results from the present longitudinal study suggest that a low serum Ca/Mg ratio is significantly associated with periodontal disease progression in Japanese elderly smokers.

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Address:
Akihiro Yoshihara
Department of Oral Health Science
Division of Preventive Dentistry
Graduate School of Medical and Dental Sciences
Niigata University
2-5274, Gakkocho-Dori
Chuo-ku, Niigata 951-8514
Japan
E-mail: akihiro@dent.niigata-u.ac.jp

Clinical Relevance

Scientific rationale for the study: Investigating the relationship of calcium and magnesium with periodontal disease is important to understand the role played by minerals in the aetiology and/or the progression of periodontal disease.

Principal findings: There was a significant dose–response relationship of Ca/Mg ratio quartiles for periodontal disease events among smokers.

Practical implications: The patients with both the long exposed years to smoking and a low serum Ca/Mg ratio have a significant high risk for periodontal disease progression.