

# Risk factors for osteoporosis in Japan: is it associated with *Helicobacter pylori*?

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**Background:** A number of diseases and drugs may influence bone mineral density; however, there are few reports concerning the relationship between lifestyle-related diseases and osteoporosis in Japan as determined by multivariate analysis. The aim of this study was to investigate the risk factors for osteoporosis and whether infection by or eradication of *Helicobacter pylori* is associated with osteoporosis.

**Methods:** Between February 2008 and November 2014, using a cross-sectional study design, we investigated patient profile (age, sex, BMI, alcohol, smoking), *H. pylori* infection status, comorbidities, internal medicine therapeutic agents (calcium channel blocker, HMG-CoA reductase inhibitors, proton pump inhibitor), serum parameters (Hb, calcium,  $\gamma$ GTP), bone turn over markers (bone-specific alkaline phosphatase (BAP) and collagen type I cross-linked N telopeptide (NTX), findings on dual-energy x-ray absorptiometry (DEXA) and upper gastrointestinal endoscopy, and Frequency Scale for the Symptoms of GERD score in consecutive outpatients aged  $\geq 50$  years at our hospital. We divided the subjects into an osteoporosis group and a non-osteoporosis group and investigated risk factors for osteoporosis between the two groups by bivariate and multivariate analyses.

**Results:** Of the 255 eligible study subjects, 43 (16.9%) had osteoporosis. Bivariate analysis showed that advanced age, female sex, lower body mass index, lower cumulative alcohol intake, lower Brinkman index, *H. pylori* positivity, lower hemoglobin, bone-specific alkaline phosphatase, lower prevalence of hiatal hernia, and endoscopic gastric mucosal atrophy were related to osteoporosis. Multivariate analysis showed that advanced age (odds ratio [OR] 1.13, 95% confidence interval [CI] 1.07–1.19,  $P < 0.001$ ), female sex (OR 6.27, 95% CI 2.26–17.39,  $P < 0.001$ ), low BMI (OR 0.82, 95% CI 0.72–0.94,  $P = 0.005$ ), *H. pylori* positivity (OR 3.00, 95% CI 1.31–6.88,  $P = 0.009$ ), and BAP (OR 1.07, 95% CI 1.01–1.14,  $P = 0.035$ ) were related to osteoporosis.

**Conclusion:** Advanced age, low BMI, BAP, and *H. pylori* positivity were risk factors for osteoporosis; however, the success of *H. pylori* eradication was not a risk factor for osteoporosis in Japan.

**Keywords:** osteoporosis, risk factor, *Helicobacter pylori*, eradication, proton pump inhibitor, lifestyle disease

## Introduction

The increase in the national cost of health care caused by the increasing numbers of patients requiring nursing care has become a serious worldwide problem in an aging society. Osteoporosis is a silent disease characterized by decreased bone mineral density (BMD) with a risk of spine and hip fractures. There are an estimated 12,800,000 patients with osteoporosis in Japan. The spine and hip fractures caused by osteoporosis are the main factors responsible for patients needing nursing care and becoming bedridden, have a significant impact on the prognosis,<sup>1</sup> and are a social burden.<sup>2</sup> Since it is expected that one third of the population of Japan will be elderly by 2030, prevention of osteoporosis is an urgent issue. However, the incidence of hip fracture continues to rise in Japan because the rate of diagnosis of osteoporosis before onset of fracture and

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awareness of risk factors for the disease are low. Intervention by physicians to diagnose osteoporosis before it becomes necessary for orthopedic surgeons to treat the resulting bone fractures is very important.

There is well established evidence regarding risk factors for bone fracture,<sup>3,4</sup> including age and sex,<sup>5,6</sup> body mass index (BMI),<sup>7</sup> alcohol,<sup>8,9</sup> and smoking.<sup>10</sup> With the Westernization of dietary habits, the number of patients in Japan with lifestyle-related diseases such as hypertension, diabetes mellitus, and hyperlipidemia has increased, and a relationship between lifestyle-related diseases and osteoporosis has been suggested. With regard to the digestive organs, several studies have pointed out that inflammatory bowel disease<sup>11–13</sup> and gastrectomy<sup>14</sup> are related to development of osteoporosis. In more recent years, *Helicobacter pylori* infection,<sup>15–20</sup> hiatal hernia,<sup>21</sup> reflux esophagitis,<sup>22–24</sup> gastric mucosal atrophy,<sup>25</sup> and proton pump inhibitor (PPI) use<sup>26,27</sup> have also been identified as possible risk factors for osteoporosis. However, there are few reports about these as risk factors for asymptomatic osteoporosis based on multivariate analysis in Japan. *H. pylori* infection is a national disease that infects approximately 80% of individuals aged 50 years or older in Japan; however, there has been no report on the relationship between the success of *H. pylori* eradication and the risk of osteoporosis.

The aim of this study was to investigate the various risk factors for osteoporosis by multivariate analysis and to determine if infection by or eradication of *H. pylori* is associated with osteoporosis in Japan.

## Materials and methods

Between February 2008 and November 2014, using a cross-sectional study design, we acquired information on consecutive outpatients aged  $\geq 50$  years at our hospital for the following: patient profile (age, sex, BMI, cumulative alcohol intake [kg], Brinkman index); *H. pylori* infection status (negative, positive, or successfully eradicated); comorbidities (type 2 diabetes mellitus and hypertension); internal medical therapeutic agents (calcium channel blockers, HMG-CoA reductase inhibitors, and PPIs); serum parameters (hemoglobin [g/dL], calcium [mg/dL], and gamma glutamyl transpeptidase [IU/L]); bone turnover markers (bone-specific alkaline phosphatase [U/L] and collagen type I cross-linked N telopeptide [NTX; nmol BCE/L]); dual-energy X-ray absorptiometry (lumbar BMD [g/cm<sup>2</sup>]; T-score, young adult mean [%], and Z-score) and lateral vertebral X-rays; findings of upper gastrointestinal endoscopy (reflux esophagitis, hiatal hernia, peptic ulcer disease, endoscopic gastric mucosal atrophy [EGA]); and score on the Frequency Scale for the

Symptoms of GERD [FSSG]). We divided the subjects into two groups (with osteoporosis and without osteoporosis) and investigated risk factors for osteoporosis between the two groups by bivariate and multivariate analyses.

BMI was calculated as body weight divided by the square of body height in meters (kg/m<sup>2</sup>). The Brinkman index score was determined by the number of cigarettes smoked per day multiplied by the number of years of smoking.<sup>28</sup> Cumulative alcohol intake was defined as the cumulative intake of ethanol (kg). *H. pylori* infection status was assessed by the <sup>13</sup>C-urea breath test and/or serum antibodies to *H. pylori*. We defined a positive result for any of these tests as being positive for *H. pylori* infection. We also defined a successful eradication result by the <sup>13</sup>C-urea breath test as negative for *H. pylori* infection at 4–8 weeks after eradication therapy. In this study, success of eradication of *H. pylori* was defined as elapse of more than one year since eradication of *H. pylori*. We defined cases taking a usual dose of calcium channel blocker, HMG-CoA reductase inhibitor, or PPI for more than a half year as users of that specific therapy. Serum bone-specific alkaline phosphatase as a biomarker of bone formation and serum NTX as a biomarker of bone resorption were investigated as bone turnover markers. BMD, T-score, young adult mean, and Z-score at lumbar vertebrae 2–4 (L2–4) were measured by dual-energy X-ray absorptiometry using a Discovery A system (Hologic, Bedford, MA, USA). We investigated for the presence of fragility fractures at the ribs and lumbar spine by lateral vertebral X-rays. The diagnosis of osteoporosis was made in accordance with the 2000 version of the Japanese diagnostic criteria published by the Japanese Society for Bone and Mineral Research.<sup>29</sup> Osteoporosis was defined as a lumbar BMD less than 70% of the young adult mean, even in those without any prevalent fragility fracture. Osteoporosis was also defined as the presence of fragility fractures in any bone in a person with a BMD of less than 80% of the young adult mean. In the diagnostic criteria for primary osteoporosis (2012 revision), Soen et al explain the relationship between the World Health Organization criteria and Japanese criteria for osteoporosis as follows. The  $-2.5$  standard deviation-based values are in close agreement with 70% young adult mean for women, but displayed some slight variability for men, although no large differences were observed.<sup>30</sup> The FSSG was investigated by a questionnaire on gastroesophageal reflux disease.<sup>31</sup> We investigated findings of upper gastrointestinal endoscopy (reflux esophagitis, hiatal hernia, peptic ulcer disease, and EGA). We defined reflux esophagitis as grade A, B, C, and D according to the Los Angeles Classification, and peptic

ulcer disease as gastric and/or duodenal ulcer, or ulcer scarring. Hiatal hernia was defined as an apparent separation of the esophagogastric junction and diaphragm impression by more than 2 cm at endoscopy. EGA was classified as C-0 (normal), C-1, C-2, C-3, O-1, O-2, or O-3 using the Kimura-Takemoto classification system,<sup>32</sup> which identifies the location of the endoscopic atrophic border. Overall, EGA was scored as 0 for C-0 type, 1 for C-1 type, 2 for C-2 type, 3 for C-3 type, 4 for O-1 type, 5 for O-2 type, and 6 for O-3 type.

We excluded patients with the following: gastrectomy, inflammatory bowel disease, chronic obstructive pulmonary disease, malignant disease (gastric, esophageal, colon, lung, pancreatic, liver, bile duct, gallbladder, breast, uterine, ovarian, prostate, or bladder cancer, or malignant lymphoma, leukemia, multiple myeloma), chronic kidney disease, type 1 diabetes mellitus, hypo/hyperthyroidism, a hypo/hyperparathyroid disorder, rheumatoid arthritis (including other collagen disease), and a premenopausal status. We also excluded patients who were currently or previously treated with agents affecting BMD and causing secondary osteoporosis, including glucocorticoids, hormone replacement therapy, thyroid/parathyroid drugs, psychotropic drugs, anticonvulsants, selective estrogen receptor modulators, vitamin D, calcium, and bisphosphonates. This study was conducted in accordance with the tenets of the Declaration of Helsinki.

## Statistical analysis

We divided the subjects into two groups (with osteoporosis and without osteoporosis) and investigated the risk factors for osteoporosis between the two groups by bivariate and multivariate analyses. Multivariate logistic regression analysis was performed using a backward selection method (likelihood ratio). The odds ratio (OR) and 95% confidence interval (CI) were also used to identify the presence and strength of any associations. Standard techniques for model checking, including the model square test, Hosmer–Lemeshow goodness of fit test, Nagelkerke  $R^2$ , and discriminant hitting ratio, were used to determine the adequacy of the multivariate logistic regression model. All statistical analyses were performed using Statistical Package for the Social Sciences version 19 software (SPSS Inc, Chicago, IL, USA). Statistical significance was inferred at  $P < 0.05$ .

## Results

The clinical characteristics of the 255 eligible cases (120 men [47.1%] and 135 women [52.9%]; mean age 63.2±8.5 [50–86] years; mean BMI 22.9±3.4) are summarized in Table 1.

**Table 1** Clinical characteristics of study patients (n=255)

<b>Patient profile</b>	
Age (years)	63.2 (±8.5)**
Sex	
Male	120 (47.1)*
Female	135 (52.9)*
BMI (kg/m <sup>2</sup> )	22.9 (±3.4)**
Cumulative alcohol intake (kg)	198 (±394)**
Brinkman index	249 (±421)**
<b>H. pylori infection status</b>	
H. pylori infection	
Negative	114 (44.7)*
Positive	94 (36.9)*
Successful eradication	47 (18.4)*
<b>Comorbidities</b>	
Diabetes mellitus	
No	221 (86.7)*
Yes	34 (13.3)*
Hypertension	
No	174 (68.2)*
Yes	81 (31.8)*
<b>Internal medical therapeutic agents</b>	
Calcium channel blocker	
Non-user	142 (82.4)*
User	45 (17.6)*
HMG-CoA reductase inhibitors	
Non-user	208 (81.6)*
User	47 (18.4)*
Proton pump inhibitor	
Non-user	183 (71.8)*
User	72 (28.2)*
<b>Serum parameters</b>	
Hb (g/dL)	13.8 (±1.4)**
Calcium (mg/dL)	9.5 (±0.4)**
γGTP (IU/L)	36.1 (±42.5)**
<b>Bone turnover markers</b>	
BAP (U/L)	22.5 (±6.3)**
NTX (nmolBCE/L)	14.1 (±5.0)**
<b>Lumbar DXA</b>	
Bone mineral density (g/cm <sup>2</sup> )	0.88 (±0.17)**
T-score	-1.32 (±1.38)**
Young adult mean (%)	85.0 (±15.5)**
Z-score	0.12 (±1.05)**
<b>Upper GI findings</b>	
Reflux esophagitis	
No	240 (94.1)*
Yes	15 (5.9)*
LA-grade A (n)	11
Grade B (n)	4
Grade C (n)	0
Grade D (n)	0
Hiatal hernia	
No	138 (54.1)*
Yes	117 (45.9)*
Peptic ulcer disease	
No	206 (80.8)*
Yes	49 (19.2)*
Gastric ulcer (n)	27
Duodenal ulcer (n)	16
Gastroduodenal ulcer (n)	6

(Continued)

**Table 1** (Continued)

Patient profile	
Endoscopic gastric mucosal atrophy	2.0 ( $\pm 1.9$ )**
C-0	72 (28.2)*
C-1	56 (22.0)*
C-2	37 (14.5)*
C-3	16 (6.3)*
O-1	37 (14.5)*
O-2	26 (10.2)*
O-3	11 (4.3)*
Questionnaire	
FSSG	7.5 ( $\pm 7.9$ )**

**Notes:** \*Number (%), \*\*Median ( $\pm$ SD).

**Abbreviations:** BAP, bone-specific alkaline phosphatase; BMI, body mass index; DXA, dual x-ray absorptiometry; FSSG, Frequency Scale for the Symptoms of GERD; GERD, gastroesophageal reflux; GI, gastrointestinal;  $\gamma$ GTP, gamma glutamyl transpeptidase; Hb, hemoglobin; *H. pylori*, *Helicobacter pylori*; LA, Los Angeles; NTX, collagen type I cross-linked N telopeptide; SD, standard deviation.

*H. pylori* negative, *H. pylori* positive, and *H. pylori* successfully eradicated cases were 114 (44.7%), 94 (36.9%), and 47 (18.4%), respectively. The mean time interval since successful eradication of *H. pylori* was  $4.4 \pm 3.7$  years and the mean age of cases with successful eradication was  $59.4 \pm 7.4$  years ( $n=47$ ). There were 34 (13.3%) and 81 (31.8%) cases of type 2 diabetes mellitus and hypertension, respectively. Calcium channel blockers, HMG-CoA reductase inhibitors, and PPIs were being taken by 45 (17.6%), 47 (18.4%), and 72 (28.2%) cases, respectively. The mean duration of PPI use among PPI users was  $3.2 \pm 3.1$  years ( $n=72$ ). Of the 255 eligible cases, 43 (16.9%) had osteoporosis (six [14.0%] men and 37 [86.0%] women). No study patient had kyphosis or fragility fractures determined by lateral vertebral X-ray. Among cases with reflux esophagitis, 11, 4, 0, and 0, respectively, had Los Angeles grade A, B, C, and D disease. Hiatal hernia and peptic ulcer disease were observed in 117 (45.9%) and 49 (19.2%) cases, respectively.

## Bivariate analysis

Results of the bivariate analysis are shown in Table 2. In the non-osteoporosis and osteoporosis groups, the mean age was  $61.8 \pm 8.2$  versus  $69.8 \pm 7.0$  years ( $P < 0.001$ ), the proportion of women was 46.2% (98/212) versus 86.0% (37/43;  $P < 0.001$ ), BMI was  $23.3 \pm 3.3$  versus  $21.1 \pm 3.3$  ( $P < 0.001$ ), cumulative alcohol intake was  $227 \pm 423$  versus  $54 \pm 121$  kg ( $P=0.015$ ), Brinkman index was  $274 \pm 429$  versus  $130 \pm 357$  ( $P=0.048$ ), those who were *H. pylori* positive comprised 32.5% (69/212) versus 58.1% (25/43;  $P=0.006$ ), those in whom *H. pylori* was successfully eradicated comprised 19.8% (42/212) versus 11.6% (5/43;

$P=0.889$ ), those with type 2 diabetes mellitus comprised 14.6% (31/212) versus 7.0% (3/43;  $P=0.190$ ), those with hypertension comprised 32.5% (69/212) versus 27.9% (12/43;  $P=0.552$ ), calcium channel blocker users comprised 16.0% (34/212) versus 25.6% (11/43;  $P=0.138$ ), HMG-CoA reductase inhibitor users comprised 17.9% (38/212) versus 20.9% (9/43;  $P=0.643$ ), and PPI users comprised 27.4% (58/212) versus 32.6% (14/43;  $P=0.490$ ). Serum values in the non-osteoporosis group compared with the osteoporosis group were  $14.0 \pm 1.3$  versus  $12.9 \pm 1.2$  g/dL for hemoglobin ( $P < 0.001$ ),  $9.5 \pm 0.3$  versus  $9.5 \pm 0.4$  mg/dL for calcium ( $P=0.138$ ), and  $37.6 \pm 45.7$  versus  $28.5 \pm 19.4$  IU/L for gamma glutamyl transpeptidase ( $P=0.198$ ). In the non-osteoporosis and osteoporosis groups, BAP was  $22.0 \pm 6.3$  versus  $24.9 \pm 5.4$  IU/L ( $P=0.007$ ) and NTX was  $13.9 \pm 5.0$  versus  $15.2 \pm 5.2$  nmol BCE/L ( $P=0.109$ ). Comparing comorbidities between the non-osteoporosis group and the osteoporosis group, reflux esophagitis was present in 6.6% (14/212) versus 2.3% (1/43;  $P=0.299$ ), respectively, hiatal hernia in 49.5% (105/212) versus 27.9% (12/43;  $P=0.011$ ), peptic ulcer disease in 17.9% (38/212) versus 25.6% (11/43;  $P=0.248$ ), and mean score of endoscopic gastric mucosal atrophy (EGA) in  $1.9 \pm 1.8$  versus  $2.7 \pm 2.2$  ( $P=0.009$ ). The median score on the FSSG questionnaire was  $7.7 \pm 8.1$  versus  $6.9 \pm 6.7$  ( $P=0.578$ ) for the non-osteoporosis group and the osteoporosis group, respectively.

## Multivariate logistic regression analysis

Models were adjusted for the parameters of age, sex, BMI, cumulative alcohol intake, Brinkman index, *H. pylori* infection status, type 2 diabetes mellitus, calcium channel blocker, PPI, hemoglobin, calcium, gamma glutamyl transpeptidase, bone-specific alkaline phosphatase, NTX, hiatal hernia, and EGA. Statistical compatibility in the multivariate analysis was as follows: model square test,  $P < 0.01$ ; Hosmer–Lemeshow goodness of fit test,  $P=0.925$ ; Nagelkerke  $R^2$ , 0.437; and the discriminant hitting ratio, 83.1%. Multivariate logistic regression showed that advanced age (OR 1.13, 95% CI 1.07–1.19,  $P < 0.001$ ), female sex (OR 6.27, 95% CI 2.26–17.39,  $P < 0.001$ ), lower BMI (OR 0.82, 95% CI 0.72–0.94,  $P=0.005$ ), *H. pylori* positivity (OR 3.00, 95% CI 1.31–6.88,  $P=0.009$ ), and high BAP (OR 1.07, 95% CI 1.01–1.14,  $P=0.035$ ) were related to osteoporosis (Table 3). PPI users tended to be associated with having osteoporosis ( $P=0.073$ ) and successful eradication of *H. pylori* was not related to osteoporosis ( $P=0.657$ ). None of the other factors examined were related to osteoporosis.

**Table 2** Association of risk factors with osteoporosis (bivariate analysis)

Covariates	Osteoporosis		Bivariate	P-value
	Non-osteoporosis 212 (83.1%)*	Osteoporosis 43 (16.9%)*		
<b>Patient profile</b>				
Age (years)	61.8 (±8.2)**	69.8 (±7.0)**	0.117	<0.001
sex				
Male	114 (53.8)*	6 (14.0)*		
Female	98 (46.2)*	37 (86.0)*	1.970	<0.001
BMI (kg/m <sup>2</sup> )	23.3 (±3.3)**	21.1 (±3.3)**	-0.212	<0.001
Cumulative alcohol intake (kg)	227 (±423)**	54 (±121)**	-0.003	0.015
Brinkman index	274 (±429)**	130 (±357)**	-0.001	0.048
<b>H. pylori infection status</b>				
<i>H. pylori</i> infection				
Negative	101 (47.6)*	13 (30.2)*		
Positive	69 (32.5)*	25 (58.1)*	1.035	0.006
Successful eradication	42 (19.8)*	5 (11.6)*	-0.078	0.889
<b>Comorbidities</b>				
Diabetes mellitus				
No	181 (85.4)*	40 (93.0)*		
Yes	31 (14.6)*	3 (7.0)*	-0.826	0.190
Hypertension				
No	143 (67.5)*	31 (72.1)*		
Yes	69 (32.5)*	12 (27.9)*	-0.22	0.552
<b>Internal medical therapeutic agents</b>				
Calcium channel blocker				
Non-user	178 (84.0)*	32 (68.3)*		
User	34 (16.0)*	11 (25.6)*	0.588	0.138
HMG-CoA reductase inhibitors				
Non-user	174 (82.1)*	34 (79.1)*		
User	38 (17.9)*	9 (20.9)*	0.192	0.643
Proton pump inhibitor				
Non-user	154 (82.6)*	29 (67.4)*		
User	58 (27.4)*	14 (32.6)*	0.248	0.490
<b>Serum parameters</b>				
Hb (g/dL)	14.0 (±1.3)**	12.9 (±1.2)**	-0.68	<0.001
Calcium (mg/dL)	9.5 (±0.3)**	9.5 (±0.4)**	-0.702	0.138
γGTP (IU/L)	37.6 (±45.7)**	28.5 (±19.4)**	-0.01	0.198
<b>Bone turnover markers</b>				
BAP (U/L)	22.0 (±6.3)**	24.9 (±5.4)**	0.071	0.007
NTX (nmolBCE/L)	13.9 (±5.0)**	15.2 (±5.2)**	0.049	0.109

(Continued)



Table 2 (Continued)

Covariates	Osteoporosis 43 (16.9%)*		Bivariate		P-value
	Non-osteoporosis 212 (83.1%)*	Standardized coefficient	OR (95% CI)	Standardized coefficient	
<b>Lumbar DXA</b>					
Bone mineral density (g/cm <sup>2</sup> )	0.93 (±0.14)**	0.64 (±0.07)**			
T-score	-0.90 (±1.06)**	-3.41 (±0.68)**			
Young adult mean (%)	89.6 (±12.4)**	62.3 (±6.5)**			
Z-score	0.31 (±1.00)**	-0.80 (±0.78)**			
<b>Upper GI findings</b>					
Reflux esophagitis					
No	198 (93.4)*	42 (97.7)*	1.00 (reference)		
Yes	14 (6.6)*	1 (2.3)*	0.34 (0.04–2.63)	-1.088	0.299
LA-grade A	10	1			
Grade B	4	0			
Grade C	0	0			
Grade D	0	0			
Hiatal hernia					
No	107 (50.5)*	31 (72.1)*	1.00 (reference)		
Yes	105 (49.5)*	12 (27.9)*	0.39 (0.19–0.81)	-0.93	0.011
Peptic ulcer disease					
No	174 (82.1)*	32 (74.4)*	1.00 (reference)		
Yes	38 (17.9)*	11 (25.6)*	1.57 (0.73–3.40)	0.454	0.248
Endoscopic gastric mucosal atrophy (EGA)					
C-0	1.9 (±1.8)**	2.7 (±2.2)**	1.25 (1.06–1.48)	0.223	0.009
C-1	61 (28.8)*	11 (25.6)*			
C-2	51 (24.1)*	5 (11.6)*			
C-3	33 (15.6)*	4 (9.3)*			
O-1	12 (5.7)*	4 (9.3)*			
O-2	29 (13.7)*	8 (18.6)*			
O-3	21 (9.9)*	5 (11.6)*			
Questionnaire					
FSSG	7.7 (±8.1)**	6.9 (±6.7)**	0.99 (0.94–1.03)	-0.013	0.578

Notes: \*Number (%), \*\*Median (±SD).

Abbreviations: BAP, bone-specific alkaline phosphatase; CI, confidence interval; BMI, body mass index; FSSG, Frequency Scale for the Symptoms of GERD; GERD, gastroesophageal reflux;  $\gamma$ -GTP, gamma glutamyl transpeptidase; *H. pylori*, *Helicobacter pylori*; LA, Los Angeles; NTX, collagen type I cross-linked N telopeptide; OR, odds ratio; SD, standard deviation.

**Table 3** Association of risk factors with osteoporosis (multivariate logistic regression analysis)

Covariates	Multivariate*		
	Standardized coefficient	OR (95% CI)	P-value
<b>Patient profile</b>			
Age (years)	0.124	1.13 (1.07–1.19)	<0.001
Sex			
Male		1.00 (reference)	
Female	1.836	6.27 (2.26–17.39)	<0.001
BMI (kg/m <sup>2</sup> )	–0.193	0.82 (0.72–0.94)	0.005
Cumulative alcohol intake (kg)	–0.001	0.99 (0.99–1.00)	0.454
Brinkman index	0.000	1.00 (0.99–1.00)	0.499
<b><i>H. pylori</i> infection status</b>			
<i>H. pylori</i> infection			
Negative		1.00 (reference)	
Positive	1.099	3.00 (1.31–6.88)	0.009
Successful eradication	0.301	1.35 (0.36–5.09)	0.657
<b>Comorbidities</b>			
Diabetes mellitus			
No		1.00 (reference)	
Yes	–0.646	0.52 (0.13–2.18)	0.374
<b>Internal medical therapeutic agents</b>			
Calcium channel blocker			
Non-user		1.00 (reference)	
User	0.635	1.89 (0.64–5.60)	0.252
Proton pump inhibitor			
Non-user		1.00 (reference)	
User	0.841	2.32 (0.93–5.81)	0.073
<b>Serum parameters</b>			
Hb (g/dL)	–0.188	0.83 (0.57–1.20)	0.319
Calcium (mg/dL)	0.067	1.07 (0.31–3.65)	0.914
γGTP (IU/L)	–0.010	1.01 (0.99–1.03)	0.176
<b>Bone turnover markers</b>			
BAP (U/L)	0.069	1.07 (1.01–1.14)	0.035
NTX (nmolBCE/L)	0.021	1.02 (0.94–1.11)	0.613
<b>Upper GI findings</b>			
Hiatal hernia			
No		1.00 (reference)	
Yes	–0.597	0.55 (0.22–1.36)	0.194
<b>Endoscopic gastric mucosal atrophy</b>			
	–0.074	0.93 (0.71–1.22)	0.593

**Notes:** \*Models adjusted for age, sex, BMI, cumulative alcohol intake, Brinkman index, *H. pylori* infection, T2DM, CCB, PPI, Hb, Calcium, γGTP, BAP, NTX, HH and EGA. Model square test  $P < 0.01$ ; Nagelkerke R<sup>2</sup> 0.437; Hosmer–Lemeshow goodness of fit test  $P = 0.925$ ; discriminant hitting ratio 83.1%.

**Abbreviations:** BAP, bone-specific alkaline phosphatase; CCB, calcium channel blocker; CI, confidence interval; BMI, body mass index; EGA, endoscopic gastric mucosal atrophy; GI, gastrointestinal; HH, hiatal hernia; γGTP, gamma glutamyl transpeptidase; *H. pylori*, *Helicobacter pylori*; NTX, collagen type I cross-linked N telopeptide; OR, odds ratio; PPI, proton pump inhibitor; SD, standard deviation; T2DM, type 2 diabetes mellitus.

## Discussion

This cross-sectional study investigated the risk of osteoporosis associated with various diseases and drugs by multivariate analysis in Japanese cases. Our results clarified for the first time that *H. pylori* infection is a risk factor for osteoporosis; however, the success of *H. pylori* eradication did not associate with osteoporosis. *H. pylori* chronically infects more than 50% of the human population, and the relationship between *H. pylori* infection and non-gastric conditions, such as lifestyle-related diseases, has recently started to attract attention.

Regarding inflammatory cytokines after eradication of *H. pylori*, Moss et al<sup>33</sup> and Yamaoka et al<sup>34</sup> reported that tumor necrosis factor-alpha and interleukin-8 significantly decreased the expression of mRNA compared to its expression before eradication. Furthermore, according to one meta-analysis, gastric mucosal atrophy of the gastric corpus and antrum was improved by successful eradication of *H. pylori*.<sup>35</sup> In Japan, it was reported that gastric mucosal atrophy was improved 10 years after eradication of *H. pylori*.<sup>36</sup> Iijima et al<sup>37</sup> reported that gastric acid secretion has a tendency to increase gradually after eradication

of *H. pylori*. Inflammatory cytokines are known to promote bone resorption<sup>38</sup> and the decrease in gastric acid secretion causes digestive and absorption disorders relating to bone metabolism, such as for calcium.<sup>39</sup> A decrease in levels of inflammatory cytokines and improvement in gastric mucosal atrophy after the success of *H. pylori* eradication may contribute to prevent the onset of osteoporosis. It was suggested that, since the mean duration after successful eradication of *H. pylori* was relatively long ( $4.4 \pm 3.7$  years) and the mean age at successful eradication was relatively young ( $59.4 \pm 7.4$  years), the improvement in BMD had may be seen in the success of *H. pylori* eradication.

In this study, we examined the relationship between various patient characteristics and osteoporosis, and found that advanced age, female sex, and low BMI were associated with osteoporosis, as in previous reports.<sup>5-7,40</sup> The prevalence of *H. pylori* infection in this study was also similar to that reported elsewhere for Japanese patients 50 years of age or older.<sup>41</sup>

With regard to the relationship between alcohol and osteoporosis, Kanis et al<sup>8</sup> reported that an alcohol intake  $>20$  g per day influenced bone density, and Berg et al<sup>9</sup> reported that excessive alcohol consumption is a risk factor for osteoporosis but that moderate drinking has a good effect on bone. In our study, there were few drinkers with a cumulative alcohol intake more than 1 kg (5.5%, 14/255), and bivariate analysis showed a lower intake of alcohol in the osteoporosis group than in the non-osteoporosis group. This result suggests that moderate drinking might favorably influence BMD.

Concerning the relationship between smoking and osteoporosis, Hopper et al reported that smoking more than 20 cigarettes per day influenced bone density.<sup>10</sup> Because the number of smokers of more than 20 cigarettes per day was low in our subjects (9.8%, 25/255), smoking may not have influenced bone metabolism in this study.

The possibility that various diseases and drugs aside from those previously known to be risk factors could influence bone density was suggested. To investigate a possible association between these diseases and drugs with BMD, we targeted subjects who did not have diseases (such as chronic kidney disease, chronic obstructive pulmonary disease, malignancy) and who did not take drugs (steroids, bisphosphonates) for which there was previous evidence of an influence on BMD,<sup>42,43</sup> and investigated the relationship between these factors and osteoporosis by multivariate analysis.

It was reported that even though the BMD of type 2 diabetes mellitus was similar with nondiabetic patients, the relative

risk of vertebral fracture was high in patients with type 2 diabetes mellitus.<sup>44</sup> A meta-analysis revealed that the relative risk of hip fracture increased in the patients with type 2 diabetes mellitus in comparison with a control group.<sup>45,46</sup> In our study, we investigated the risk of osteoporosis, but not bone fracture, in type 2 diabetes mellitus, and found that type 2 diabetes mellitus was not a risk factor for osteoporosis, as reported previously. However, evaluation of risk factors for bone fracture in patients with type 2 diabetes mellitus may be clinically important in the future.

With regard to hypertension, Cappuccio et al<sup>47</sup> reported that systolic blood pressure is related to femoral BMD. Tsuda et al<sup>48</sup> suggested that high blood pressure might be associated with reduced BMD in females with untreated hypertension. In the association between antihypertensive agents and bone metabolism by meta-analysis, Wiens et al reported that thiazide diuretics and beta-blockers appeared to lower the risk of fractures.<sup>49</sup> There has not been a large-scale clinical study of the relationship between use of calcium channel blockers and osteoporosis. The relationship between hypertension, antihypertensive agents, and osteoporosis was not seen. The limitations were: no comparison between those having treatment and those not having treatment in patients with hypertension; we only investigated calcium channel blocker use among antihypertensive agents, therefore the relationship between hypertension, antihypertensive agents and osteoporosis might not be detected.

HMG-CoA reduces cholesterol composition by inhibiting the mevalonate metabolic pathway. Because inhibition of bone resorption is related to inhibition of this pathway, HMG-CoA may contribute to the inhibition of bone resorption.<sup>50</sup> Chung et al reported that BMD in users of HMGCoA reductase inhibitors was increased in comparison with that in nonusers in a large-scale clinical study.<sup>51</sup> On the other hand, Rejnmark et al reported that HMGCoA reductase inhibitors did not change BMD at the lumbar and femoral vertebrae.<sup>52</sup> It is thought that there is not sufficient evidence to suggest an action of HMGCoA reductase inhibitors on bone, and in this study a clear association between use of HMGCoA reductase inhibitors and osteoporosis was not found.

PPIs are first-line therapy for gastroesophageal reflux disease and are the most popular gastric acid secretion inhibitors worldwide. Since ionization of calcium with gastric acid is affected by strong suppression of gastric acid secretion, it is thought that osteoporosis is caused by digestive and absorption disorders involving calcium.<sup>53</sup> On the other hand, it is thought that PPIs inhibit bone resorption by inhibiting the proton pump in osteoclasts. In recent years, several studies



have reported that high-dose PPI is a risk factor for hip fracture;<sup>26,27</sup> however, these reports were retrospective in nature. In our cross-sectional study the multivariate analysis revealed that PPI use showed a tendency ( $P=0.073$ ) toward a relationship with osteoporosis; however, as in the results of a large study in Western countries,<sup>26,54</sup> the relationship between PPI use and osteoporosis remains controversial, and a multicenter prospective study including a large number of cases will be necessary.

There are various reports on the association between gastroesophageal reflux disease and osteoporosis. In previous reports, subjects were often already complicated by multiple vertebral fractures.<sup>21–24</sup> In this study, conducted in the setting of internal medicine in an urban area, no case was complicated with vertebral fracture or kyphosis. In addition, results from the FSSG symptom questionnaire on gastroesophageal reflux disease did not differ between groups. Further, in bivariate analysis there was a low prevalence of hiatal hernia in the osteoporosis group compared with that in the non-osteoporosis group. The non-osteoporotic patients often had hiatal hernia and were negative for *H. pylori*. This result is consistent with a previous report that patients with reflux esophagitis often had hiatal hernia and were negative for *H. pylori*.<sup>55</sup>

Sawicki et al reported that peptic ulcer disease was an independent risk factor for osteoporosis.<sup>56</sup> In patients with peptic ulcer disease, the gastric and duodenal epithelium are defective, and inflammation at these sites may cause malabsorption of calcium and other substances, such as macroelements, that play a crucial role in mineral homeostasis and bone metabolism.<sup>57</sup> Our results demonstrated that peptic ulcer disease was not a risk factor for osteoporosis, and disorders of the small intestine may contribute more to absorption of various nutrients than disorders of the stomach and duodenum.

The decrease in dissolution of calcium salts by the decrease in gastric acid secretion in patients with atrophic gastritis may also result in malabsorption of calcium.<sup>39</sup> Kim et al<sup>25</sup> reported that atrophic gastritis was associated with an increased likelihood of osteoporosis. Results for EGA in our data did not correlate with osteoporosis in multivariate analysis, and it was suggested that EGA was a confounding factor of *H. pylori*.

With regard to markers of bone metabolism, it was shown that bone-specific alkaline phosphatase was related to osteoporosis. The osteoporosis patients in this study were considered to be young (mean age  $69.8 \pm 7.0$  years), and 86.0% (37/43) of the cases were female, suggesting that most had the high turnover type of osteoporosis. NTX was not significantly related to osteoporosis in our study. The

circadian rhythm of NTX activity may have influenced the result of blood examination.

## Conclusion

This cross-sectional study revealed various diseases and drugs as risk factors for osteoporosis by multivariate analysis of Japanese cases and clarified for the first time that *H. pylori* infection is a risk factor for osteoporosis. However, the success of *H. pylori* eradication is not associated with the risk of osteoporosis. Because *H. pylori* infection is a national disease in Japan, affecting approximately 80% of those aged 50 years or older, and osteoporosis is a serious disease causing hip fracture and affecting the lifespan, we believe that we should be providing medical care in consideration of these risk factors for osteoporosis in clinical practice.

This study has some limitations. The data were collected from outpatients at a university hospital (a single-center trial) in an urban community in Japan. The study participants were not a random sample of subjects, and we did not investigate their family history of osteoporosis or certain other important risk factors, such as exercise, sunlight exposure, dietary calcium intake, or other nutrients. Further, the number of participants in this cross-sectional study was not very large, so a large prospective multicenter trial will be required.

## Disclosure

The authors report no conflicts of interest in this work.

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