BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-027356
Article Type:	Research
Date Submitted by the Author:	18-Oct-2018
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Keywords:	osteoporosis, bone mineral density, Helicobacter pylori, meta-analysis

Original article

Relationship between Helicobacter pylori infection and risk of osteoporosis: A systematic review and meta-analysis

Running Title: Relationship between *H. pylori* and osteoporosis

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Word count: 3003

Abstract

Objective Many studies have explored the association between Helicobacter pylori (*H. pylori*) infection and osteoporosis. However, evidence on *H. pylori* infection and risk of osteoporosis is still controversial. Therefore, we performed this systematic review and meta-analysis to evaluate the association between *H. pylori* infection and osteoporosis.

Design Systematic review and meta-analysis of case-control study.

Participants People with or without osteoporosis.

Data sources A comprehensive literature search was performed on PubMed, Embase, Web of Science and CBM for studies investigating the association between Helicobacter pylori infection and osteoporosis up to April 30, 2018. English and Chinese languages papers were considered. Two independent investigators selected studies. The methodological quality of the studies was assessed using the Newcastle–Ottawa scale.

Main outcomes and measures Pooled odds ratios (ORs) and 95% confidence intervals (95%CIs) were calculated using random effects model if heterogeneity existed, otherwise using fixed effects model was used. Subgroup analyses were also performed to explore source of the heterogeneity. Sensitivity analyses and publication bias were also tested.

Results A total of 21 studies with 9655 participants were included in our analyses. Taking together, we found that *H. pylori* infection was significantly associated with increased risk of osteoporosis (OR (95%CI), 1.39(1.13–1.71)); there was no significant difference between osteoporosis and osteopenia; males had relatively higher risk than females. However, the decrease of bone mineral density in *H. pylori* positive patients was

not significant compared with *H. pylori* negative controls, which may due to the sample size.

Conclusion Our meta-analysis suggests a significant increased risk of osteoporosis in patients with *H. pylori* infection. The clinicians should pay more attention to the patients infected with *H. pylori*. Further researches are needed to confirm these findings and to identify the underlying biological mechanisms and confounding factors, and to detect the influence of variables across studies.

Keywords: osteoporosis; bone mineral density; Helicobacter pylori; meta-analysis.

Strengths and limitations of this study

- We explored the association between osteoporosis and Helicobacter pylori, and found a positive result which is different from the previous one and more comprehensive.
- The number and quality of studies included is limited. Therefore, the results of the meta-analysis should be interpreted with caution.
- Causality can't be established in observational study.

Introduction

Helicobacter pylori (*H. pylori*), a Gram-negative and spiral-shaped bacterium dwelling on the gastric epithelium, has an influence on approximately 50% of the global population, especially those living in developing countries[1]. The prevalence of *H. pylori* infection is approximately 30% in developed countries and up to 80% in developing countries[2 3], and up to 90% in patients with dyspepsia[4]. In north Europe and North America, about one-third of adults are infected, and in south and east Europe, South America, and Asia, the prevalence of *H. pylori* is often higher than 50%[5]. Moreover, infected subjects born abroad (first-generation immigrants) had a higher risk of *H. pylori* infection than second-generation immigrants in a multi-ethnic European city [6]. Compared with high prevalence, the low spontaneous eradication rate of *H. pylori* infection makes the situation more serious. For example, the spontaneous eradication rate is only 2.9% over the one-year period among schoolchildren aged 7-12 years[7]. *H. pylori* has been well-known to associated with gastrointestinal diseases, such as gastritis, gastric ulcer, stomach cancer and so on[8]. However, many non-gastrointestinal diseases may also have associations with *H. pylori*, some of them have been proven by large-scale population researches or meta-analysis, such as preeclampsia[9], autoimmune thyroid diseases[10], myocardial infarction[11], hepatic encephalopathy[12] and prostatitis[13].

Osteoporosis is one of the most common metabolic bone diseases, characterized by decreased bone mineral density (BMD), increased bone fragility, and then increased susceptibility to fracture[14], especially in spine and hip. Osteoporosis has become a major

health concern for both individuals and societies. Osteoporosis has huge adverse impact on life quality and is associated with increased morbidity rate. The in-hospital mortality rate is also up to between 0.85 to 2.26%[15]. In Europe, about half of women and one-fifth of men aged over fifty years develop pathological hip, spine, forearm, or humerus fracture due to osteoporosis during their remaining lifetime[16]. The same situation happens in other countries or district, such as Japan[17 18] and Taiwan.

There are well established evidence regarding the risk factors for osteoporosis [18], such as age, sex, body mass index (BMI), alcohol, and smoking. *H. pylori* infection can induce individual inflammatory and immune reactions, such as the increased releasing of IL-1 and TNF-α, which could trigger bone resorption, and regulate bone turnover[19]. Recently, many studies about the association between osteoporosis and *H. pylori* have been performed. However, the role of *H. pylori* in osteoporosis remains controversial. This issue has been discussed in previous meta-analysis [20 21], but no significant association was found. As more studies evaluating the association between *H. pylori* infection and osteoporosis have been published since then [2 22-28], we carried out this updated meta-analysis to further evaluate the association between *H. pylori* infection and osteoporosis qualitatively, and the quantitative alterations of BMD in *H. pylori* infected patients compared with those in healthy controls.

Materials and Methods

This study was performed based on Preferred Reporting Items for Systematic Reviews

(PRISM) checklist [29] for systematic review and meta-analysis. Study searching and selection, quality assessment, and data extraction were done by two researchers independently to avoid bias.

Search strategy

We searched through the databases of PubMed, EMBASE, Web of Science and Chinese Biomedical Literature Database (CBM). The last search for all databases was updated to April 30, 2018. We used the combined method of MeSH Term and free words by applying the following terms: Helicobacter pylori, *campylobacter pylori, H. pylori, hp, helicobacter, helicobacter bill, helicobacter hepaticus, helicobacter pullorum, helicobacter species, helicobacter sp, helicobacter genus, campylobacter, campylobacter infection, campylobacteriosis,* Helicobacter pylori infection, Helicobacter infection, *pylori, enterohepatic helicobacter spp, campylobacter sp* and fragility fracture, bone density, bone mass density, osteocalcin, bone loss, osteoporosis. Two authors evaluated potential publications by checking their titles and abstracts and then procured the most relevant publications for a closer examination. Bibliographies section of retrieved articles were also reviewed for additional pertinent studies which were possibly missed in the initial search.

Studies selection and data Extraction

Studies were included if they met the following criteria: (1) it is an observational study; (2) its objective of interest is to assess the association between *H. pylori* infection and osteoporosis; (3) they either provided risk estimates with odds ratios (ORs) and 95%

confidence intervals (95%CIs), or sufficient information was available to calculate the ORs and 95%CIs. Articles were excluded if they were duplicate publications, reviews, animal studies, editorials, and case reports. The papers were also excluded if no effect estimate was reported or no enough raw data for ORs and 95%CIs calculation. In the case of multiple studies with the same or overlapping data published by the same researchers, we selected the most recent study with the largest number of participants. All papers meeting the criteria defined above were included for further analysis.

The literatures included were carefully reviewed for information about the first author, publication year, country, population, sample size, sex, age, detection methods of *H. pylori* and osteoporosis, diagnosis location, diagnosis, and adjusted covariates.

If data could be acquired from the tabulated literature search results, they would be extracted carefully into 2x2 tables from all eligible publications by two independent reviewers. If data were not directly available, they would be calculated from published positive predictive values and/or negative predictive values if appropriate. The adjusted OR (95%CI), if existed, was adopted instead of rude OR (95%CI). Two researchers conducted data extraction independently. A third researcher was consulted when there were discrepancies in the data, and agreement was reached after discussion.

Quality assessment

Quality assessment was performed using the Newcastle-Ottawa quality assessment scale [30]. Two researchers conducted blinded quality assessment of the included literatures. The NOS assigns a maximum of 9 points to studies of highest quality according to three

quality parameters: selection, comparability, and outcome. When the researchers' assessments were discrepant, a third researcher was consulted for the final grading.

Statistical analyses

The primary measures were ORs and 95%CIs for the association between *H. pylori* and osteoporosis, and standardized mean difference (SMD) for BMD alterations between *H. pylori* infected patients and healthy controls. To assess heterogeneity among the studies, we calculated the Cochran's Chi-squared test (with $p < 0.10$ indicating statistically significant heterogeneity) and the statistic I^2 [31] (The heterogeneity might not be important with I^2 of 0 to 40%, while moderate heterogeneity with I^2 of 30 to 60%, substantial heterogeneity with I^2 of 50 to 90% and considerable heterogeneity with I^2 of 75 to 100%). The pooled results were calculated using fixed effects model if no obvious heterogeneity existed, otherwise random effects model was used. The cumulative meta-analysis was conducted for the extracted data using a pooled random effects model with the publication year. In the event of obvious heterogeneity, subgroup analysis was performed according to sex, diagnosis method and locations of osteoporosis, and detection method of *H. pylori*. Meta regression was also performed to explore the potential heterogeneity. Publication bias was assessed by funnel plot and Egger's test (Egger's test was done only if the studies number was no less than 10) [31]. All statistical analyses were performed using Stata 12.0.

Results

Search results

Using our search criteria, a total of 1720 articles were identified through PubMed, Web of Science, Embase and CBM, and one additional record identified through other sources. Then, 443 duplicate papers were removed firstly, and 1242 papers were excluded after scanning their titles and abstracts. After screening the full texts of the included articles, 15 studies were excluded for the following reasons: duplicate publication(n=4), not about the topic(n=2), no related data about *H. pylori* and/or osteoporosis(n=4), lack of access to full text (n=5). A total of 21 studies [2 17 22-28 32-43] were included for further analysis (Fig. 1).

Study Characteristics

A total of 21 articles were included in this study. Of them, 20 provided data for association between *H. pylori* and osteoporosis[2 17 22-28 32-40 42 43], 4 for the BMD alterations in *H. pylori* positive patients compared with healthy controls[24 33 34 41], 3 provided both[24 33 34]. All these studies were published from 2005 to 2018. Four studies were conducted in China, 3 in Iran, 2 in Italy, 9 in Japan, 1 in Brazil, 1 in Korea and 1 in Turkey. As to the sex of participants, 4 were postmenopausal women, 4 were females, 4 were males, 9 involved both males and females. The detection methods of *H. pylori* were mainly ELISA and ^{13}C-urea breath test, while the detection methods of osteoporosis were dual-energy X-ray absorptiometry (DEXA) and quantitative ultrasound. As to the diagnosis, 4 were osteopenia, 14 were osteoporosis, 1 provided both, and 1 provided decreased BMD (treated as osteopenia for analysis) (Table 1). In addition, 13 studies

showed no significant associations of *H. pylori* infection and osteoporosis, while 8 showed significant associations.

Quality evaluation

The Newcastle-Ottawa scale was adopted to evaluate the quality of these case-control studies. Among the selection items, the evaluation results ranged from 4 to 8 stars, with the median NOS score was 6, indicating a medium quality of the studies included. The most common source of bias mainly happened in selection and comparability. (Table 1)

Synthesis of the results

As shown in Fig. 2, the overall OR was obtained based on the 20 studies involving the *H. pylori* and osteoporosis (including osteopenia) (a total of 8788 patients and healthy controls). The pooled OR and its 95% CI were 1.39 (1.13,1.71), indicating *H. pylori* infection was significantly associated with increased risk of osteoporosis/osteopenia. A cumulative meta-analysis was conducted with publication year in ascending order, and the results indicated that the pooled OR (95% CI) started to show statistical significance at 1.57 (95% CI: 1.02,2.41) from the ninth analyzed study, with gradually stabilizing results afterwards.

Subgroup analyses

Giving that obvious heterogeneity existed, subgroup analyses were performed based on the factors of potential heterogeneity. All 20 studies were involved in these subgroup analyses. As one study reported both osteoporosis and osteopenia, it was used in two groups based on the data. Fig 3 showed that both osteoporosis and osteopenia were

significantly associated with *H. pylori* infection with OR (95%CI) of 1.59(1.16, 2.20) and 1.21(1.03, 1.43) respectively. Although the OR was a little higher in osteoporosis group, the meta regression analysis showed no significant difference between these two groups (beta=0.37, t=1.37, p=0.18). Therefore, we pooled osteoporosis and osteopenia together to analyze other confounding factors.

Results of subgroup analyses by other risk factors were shown in Table 2. We found that males had a little higher risk than females, but did not reach the significant level. No significant risk was found in females in the subgroup analysis by whether menopause or not. In different countries, we found significant associations between *H. pylori* infection and osteoporosis in China, Japan, Korea (three East Asian countries). Other factors that may affect the results were presented in Table 2.

Publication bias and sensitivity analyses

Begg's test and Egger's test were employed to examine the pooled values from five or more studies, and both indicated no publication bias in any of the analyzed data (Fig 4). Sensitivity analysis was conducted for the pooled results by removing any single trial and by converting the pooled model (fixed effects model). The overall result didn't change significantly by removing any single trial or converting the pooled model, which showed stable results.

Alterations of BMD in H. pylori infected population

Four studies were involved in this meta-analysis[24 33 34 41]. As each has more than one subgroup data from different DEXA detection locations, we carried out the subgroup

analysis based on detection location. As shown in supplementary fig 1, all three subgroups' and overall results had no significant alterations in BMD.

Discussion

Although osteoporosis isn't a deadly disease, osteoporosis causes huge burden to individuals and society owing to its high morbidity. Here, we got a comprehensive result by meta-analysis, indicating that *H. pylori* infection may be a risk factor for osteoporosis. However, the mechanism is still unclear. Several possible mechanisms may explain this result. First, *H. pylori* infection may lead to systemic inflammation, and release of cytokines, such as tumor necrosis factor-alpha, interleukin-1 and interleukin-6[44], which may cause bone turnover indirectly. Second, many studies have shown that lower vitamin B12 can be associated with *H. pylori* infected subjects[45]. As the folate becomes trapped as methyltetrahydrofolate and then interrupts for folate-related DNA synthesis, it is an important factor for bone remodeling, the low level of vitamin B12 may result in decreased BMD and osteoporosis. Third, *H. pylori* infection may decrease the calcium absorption by causing the gastric mucosal atrophy and decreasing acid secretion. Thus, eradication of *H. pylori* may increase calcium absorption and stop the process of osteoporosis through decreasing the levels of inflammatory cytokines and improving gastric mucosal atrophy.

The present meta-analysis of 20 studies indicated that patients with *H. pylori* infection were associated with an estimated 1.39 times higher risk of developing osteoporosis as

compared with those without *H. pylori* infection. Compared with the previous meta-analysis [20 21] (one had 5 studies involving 1321 participants, and one had 4 studies involving 520 participants), we had 20 studies involving 8788 participants for the association between *H. pylori* infection and osteoporosis with a pooled OR of 1.39 (1.13,1.71). Thus, the power of our result was increased, and the reliability was higher too. Other studies of *H. pylori* infection and osteoporosis risk failed to find an association, which may due to the sample size. In the quantitative analysis, the alteration of BMD was not significant, with an overall SMD (95%CI) of -0.63(-1.52,0.25), which may due to the sample size, as the result had a tendency of decrease. Therefore, more studies with large sample size are still needed to verify the alterations of BMD in *H. pylori* infection.

Despite the significant association between *H. pylori* infection and osteoporosis, obvious heterogeneity existed. We found that sex of participates may affect the results. Males had higher risk to develop osteoporosis than females when infected with *H. pylori*. However, only 7 studies (4 were about postmenopausal women and 3 were about non-postmenopausal women) were conducted in female, the results may be not that reliable due to the small sample size. Another reason may also be possible, that the different degree of osteoporosis may affect the diagnosis and some early patients may be regarded as healthy controls. Further studies with dose-response relationship of different severity of osteoporosis and prevalence may help to confirm this hypothesis. In the subgroup analysis by criteria (osteoporosis and osteopenia), the OR in osteoporosis was a little higher than that in osteopenia, which may also help to prove our hypothesis. In the

subgroup analysis based on countries, significant association was evidenced in three East-Asian countries (China, Japan, Korea), indicating many other factors that were associated with geography may affect the results.

In our research, we also explored the heterogeneity from diagnosis methods factors. We found that the detection methods of osteoporosis (DEXA and quantitative ultrasound) affected the pooled results, and the detection locations of DEXA also contributed to the heterogeneity. The same situation also happened in the detection methods of *H. pylori*. We found ELISA and multi-method strategy may provide more homogeneous results. In total, as the heterogeneity still existed obviously, further more studies were still needed.

We also compared the quantitative alterations of BMD in *H. pylori* infected subjects. However, no significant difference was found. The reason may be that: 1) the sample size was relatively small, 2) the severities of osteoporosis were not serious, or the infection of *H. pylori* didn't last long enough to cause alterations, 3) though the basic characteristics of included studies were comparable, many confounding factors that might affect BMD have not been adjusted.

The strength of the present meta-analysis lies in inclusion of 21 observational studies reporting data on *H. pylori* infection and osteoporosis, and the alterations of BMD by *H. pylori*. However, our meta-analysis has several limitations that should be recognized when interpreting the results. First, most of the included studies were hospital-based or health center-based, which were not affected by detection bias, but might be subjected to selection bias. However, the prevalence of *H. pylori* infection in most studies that we

selected was consistent with the incidence rate in the general population. Second, our analysis had an ascertainment bias that might be present because progression of osteoporosis is continuous, and some patients may be classified as controls. However, this may lead to a more conservative result, which may help to indicate that our overall result is reliable. Third, the heterogeneity is still obvious. However, we performed subgroup analyses based on study characteristics, and found that some factors may affect the association and some may not. Four, the qualities of included studies were medium, and some studies were published informally. We also included all these studies based on inclusion and exclusion criteria to avoid publication bias. However, our study is still the most comprehensive result about the association between *H. pylori* positive and osteoporosis so far.

In summary, our results suggest a significant increased risk of osteoporosis in patients with *H. pylori* infection. The clinicians should pay more attentions to the patients infected with *H. pylori*, especially those chronic gastritis patients. However, the result should be cautiously interpreted due to the inclusion of underpowered studies. Further large prospective studies are still needed to address the association between *H. pylori* infection and osteoporosis and its potential confounding factors.

Funding

This work was supported by the National Natural Science Foundation of China [No. 81202220 to Y. Z.; No. 81673211 and 81372952 to T. C.], by the Natural Science Foundation of Jiangsu Province, China [No. BK20150094 to Q.Z.], and by the Chongqing Health and Family Planning Commission [No. 20141027 to H. X.].

Contributors

YZ and HX led the study by designing, interpreting results, and revising manuscript critically for important intellectual content; TW and XL contributed to data analysis, result interpretation and drafting of the manuscript; QZ and YL participated in study data collection and revising manuscript; TW, XL, TC and YZ participated in study conduct and results interpretation. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Patient consent Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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Figure legend

Fig 1. Flow diagram of the article selection for systematic review.

Fig 2. Meta-analysis of overall OR (random effects models). Left, standard technique; Right, cumulative technique.

Fig 3. Subgroup meta-analysis according to diagnosis (random effects models).

Fig 4. Funnel plot (publication bias assessment plot) of the odds ratio.

Supplementary figure 1. Meta-analysis of SMD according to detection locations.

Table 1. Characteristics and quality assessment of the included studies on Helicobacter pylori infection and risk of osteoporosis.

Author	Year	Population	Sex(M/F)	Age(mean age±SD or (range age) years)	Detection method of H.pylori	Detection methods of osteoporosis	Diagnosis locations	Diagnosis	Total	Scores of NOS	Adjusted factors
Figura, N.	2005	Italy	Males	65 (55–82) for patients; 64.5(55–80) for controls	ELISA	DEXA	Lumbar and femur bone	Osteoporosis	240	7	Patients and controls were comparable on age, socioeconomic background, and smoking habits.
Adriana M. KAKEHASI	2007	Brazil	Postmenopausal women	61.6±7(50–79)	non-ELISA	DEXA	Lumbar spine	Osteoporosis	50	6	mean age, body mass index, age at menarche, or postmenopausal period
Adriana M. Kakehasi	2009	Italy	Females	Not mentioned	non-ELISA	DEXA	Lumbar spine and hip	Not fitted*	61	-	Age, Postmenopausal time, BMI
Akkaya, Nuray	2011	Turkey	Postmenopausal women	65.29±6.09 patients; 63.57±6.53 controls	ELISA	DEXA	Lumbar and femur neck	Osteoporosis	105	6	age, education level, occupation, age of menarche or menopause, duration of postmenopausal, period or daily consumption of tea, coffee, alcohol or dairy products
Chinda, D.	2013	Japan	379/631	not mentioned	ELISA	QU	Calcaneal osteo	Osteopenia	1010	7	multivariate Logistic Regression analysis
Asaoka, Daisuke	2014	Japan	95/105	63.1±8.8 years	Both	DEXA	Lumbar vertebrae	Osteoporosis	200	6	Multivariate Logistic Regression Analysis: age, gender, BMI, alcohol consumption, smoking, H. pylori infection, BAP, PUD, and EGA
Asaoka, D.	2014	Japan	131/26	71.1±7.5 patients 61.6±8.9 controls	not mentioned	DEXA	Lumbar	Osteoporosis	157	6	multivariate Logistic Regression analysis (age, sex, BMI, etc.)
Lin, S. C.	2014	China	Female	77 (65–97)	non-ELISA	DEXA	Not mentioned	Osteoporosis	365	5	multivariate logistic regression analyses (age group, body mass index group, and use of proton pump inhibitor, etc.)
Asaoka, D.	2015	Japan	130/134	69.8±6.8 for patients 1.9±8.2 for controls	not mentioned	DEXA	Not mentioned	Osteoporosis	264	7	multivariate analysis (age, sex, BMI etc.)
Asaoka, D.	2015	Japan	120/135	63.2±8.5	Both	DEXA	Lumbar vertebrae	Osteoporosis	255	6	Multivariate analysis (age, sex, BMI, BAP, Comorbidities etc.)
Chung, Y. H.	2015	Korea	Men	54.4± 10.7 for Hp+ 51.9± 12.1 for Hp-	ELISA	DEXA	Lumbar (L1–L4)	Osteoporosis, osteopenia	1126	7	unadjusted
Fotouk-Kiai, M.	2015	Iran	575/392	68.3±6.8 for hp+ 69.3±7.4 for hp-	ELISA	DEXA	Lumbar vertebra and Femur	Osteoporosis	967	5	Age, sex, smoking, alcohol consumption and BMI
Mizuno, S.	2015	Japanese	Men	62.1±5.0 for low TBD; 58.4±5.7 for normal	ELISA	QU	Not mentioned	Osteoporosis	230	8	Age- and BMI-adjusted
Chinda, D.	2016	Japan	Men	50.2±15.4 years	ELISA	QU	Not mentioned	Osteopenia	295	7	multivariate logistic regression analyses: age, BMI, serum level of estradiol, the intake of calcium per day, smoking, drinking, periodical exercise, last educational background

Chinda, D.	2016	Japan	Females	52.2±15.2	ELISA	QU	Not mentioned	Osteopenia	473	6	multiple logistic regression models: age, BMI, smoking, alcohol consumption, periodical exercise, last educational level, serum level of estradiol, calcium intake per day
Kalantarhormozi, M. R.	2016	Iran	Postmenopausal women	58.87±8.02	ELISA	DEXA	Not mentioned	Osteoporosis	250	6	not mentioned
Zhang, Chaoxian	2016	China	194/126	38.32±10.64 for patients: 38.27±7.46 for controls	non-ELISA	DEXA	Not mentioned	Osteoporosis	320	5	Multiple logistic regression models some basic characters and genes
Abdolahi, N.	2017	Iran	Postmenopausal women	not mentioned	ELISA	not mentioned	Not mentioned	Osteoporosis	107	8	not mentioned
Chinda, D.	2017	Japan	Females	62.5±8.6 for patients 44.9±10.9 for controls	ELISA	QU	Calcaneus	Osteopenia	473	4	Multiple logistic regression analysis: Age, BMI, Smoking, Alcohol, Exercise habit, Schooling duration, Estradiol levels, Birth history Calcium intake
Lu, Li-juan	2018	China	1474/393	54.0±9.6	non-ELISA	QU	Calcaneus	Osteoporosis	1867	6	not mentioned
Pan, B. L.	2018	China	568/299	55.9±11.3	non-ELISA	DEXA	Not mentioned	Decreased BMD	867	5	Multiple stepwise logistic regression analysis: Sex, BMI, Waist circumference, Total cholesterol, HDL, TG, LDL, Peptic ulcer, GERD

Not fitted *: this study only explored the alteration of BMD in patients with *H. pylori* infection.

ELISA: enzyme linked immunosorbent assay; QU: quantitative ultrasound; DEXA: dual energy X-ray absorptiometry scan;

Table 2. Overall effect estimates for Helicobacter pylori infection and osteoporosis risk according to study characteristics

Factors	Categories	No. of studies	OR [95%CI]	Heterogeneity	
				I^2	p-value
Sex				34.9%	P > 0.05
	Male	5	1.37(1.15,1.62)	24.6%	P > 0.05
	Female	8	1.09(0.87,1.34)	33.0%	P > 0.05
Postmenopausal or not					
	Postmenopausal women	4	1.05(0.64,1.72)	35.8%	P > 0.05
	Non-postmenopausal women	3	1.21(0.74,1.97)	57.3%	P > 0.05
Country					
	China	4	1.90(1.11,3.24)	89.0%	P < 0.05
	Japan	9	1.57(1.08,2.28)	63.7%	P < 0.05
	Italy	1	0.87(0.50,1.53)	-	-
	Brazil	1	0.42(0.12,1.42)	-	-
	Korea	1	1.43(1.17,1.74)	-	-
	Iran	3	1.06(0.60,1.86)	61.3%	P > 0.05
	Turkey	1	0.95(0.53,1.69)	-	-
Detection methods of *H. pylori*					
	ELISA	11	1.08(0.89,1.32)	46.3%	P < 0.05
	Non-ELISA	5	1.62(0.96,2.72)	87.0%	P < 0.05
	Both	2	3.67(1.88, 7.16)	0%	P > 0.05
Antibody of ELISA					
	IgA	2	1.40(0.78,2.48)	9.8%	P > 0.05
	IgG	2	1.07(0.55,2.07)	0%	P > 0.05
Detection methods of osteoporosis					
	DEXA	13	1.60(1.16,2.21)	79.6%	P < 0.05
	QU	6	1.11(0.95,1.29)	0%	P > 0.05
Detection location of DEXA					
	Lumbar	6	1.50(1.18,1.91)	65.4%	P < 0.05
	Femur	3	1.56(1.17,2.08)	0%	P > 0.05

ELISA: enzyme linked immunosorbent assay; QU: quantitative ultrasound; DEXA: dual energy X-ray absorptiometry scan.

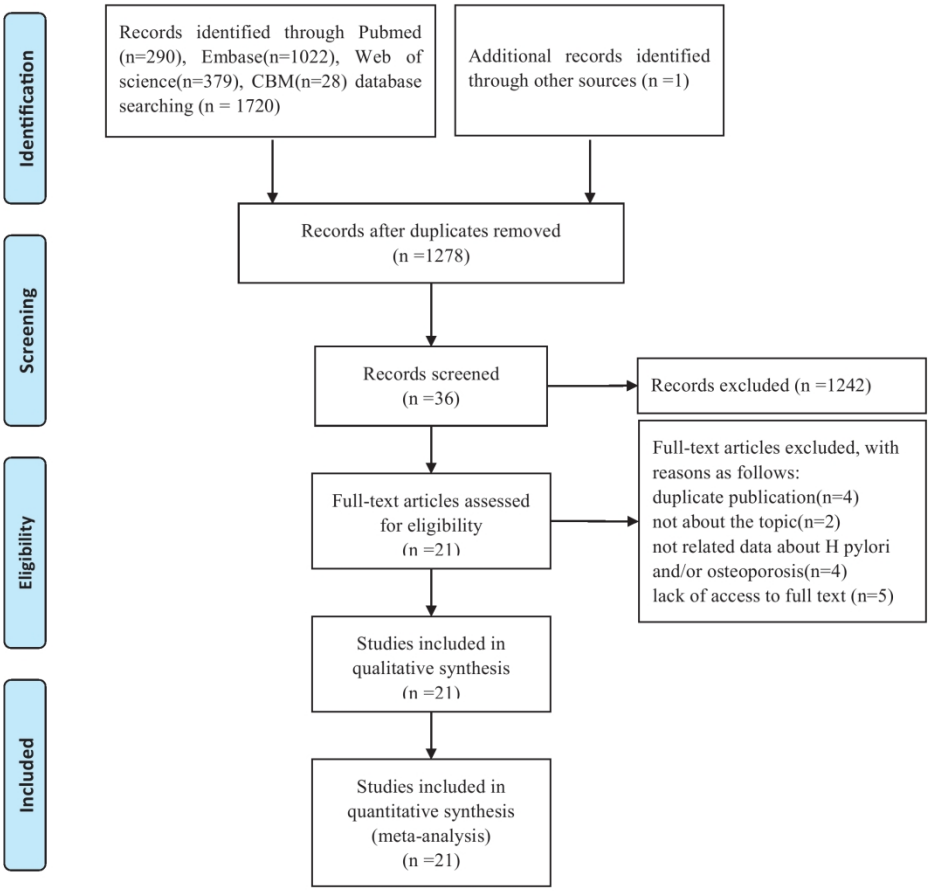

Fig 1

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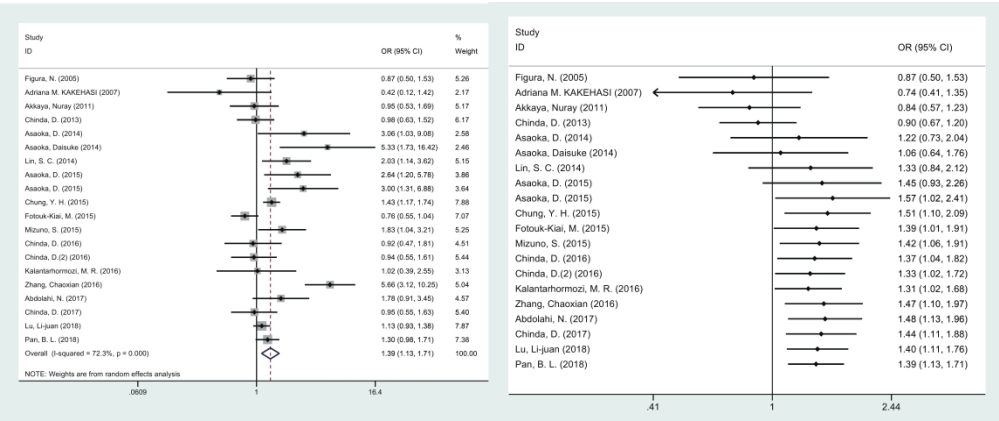

Fig 2

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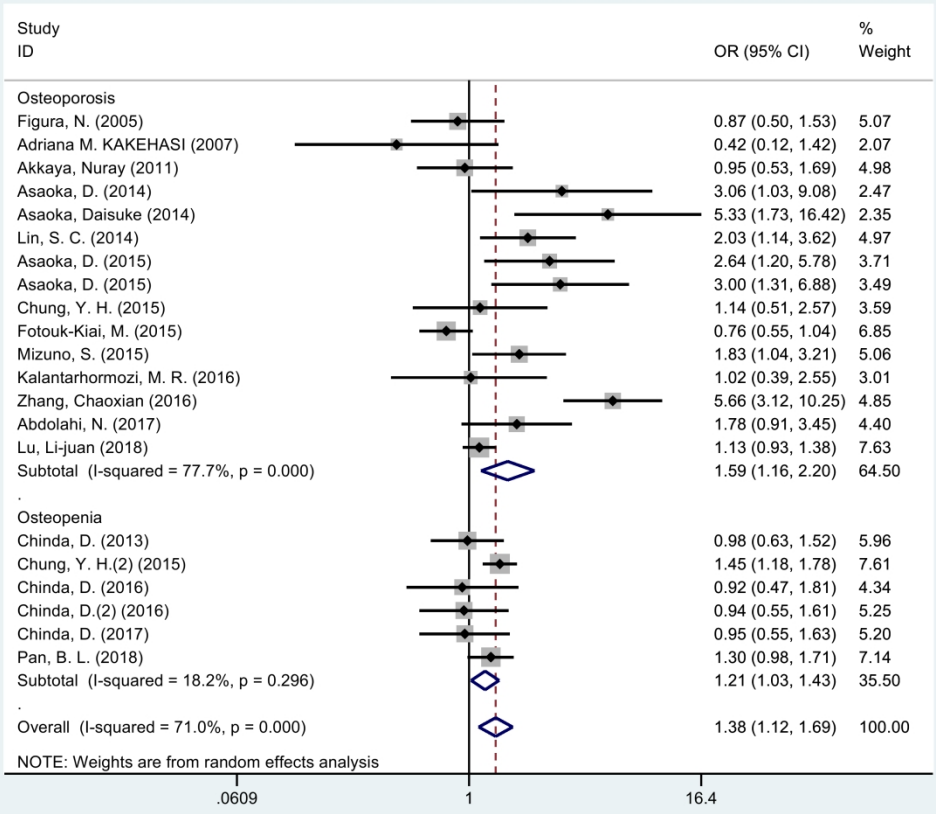

Fig 3

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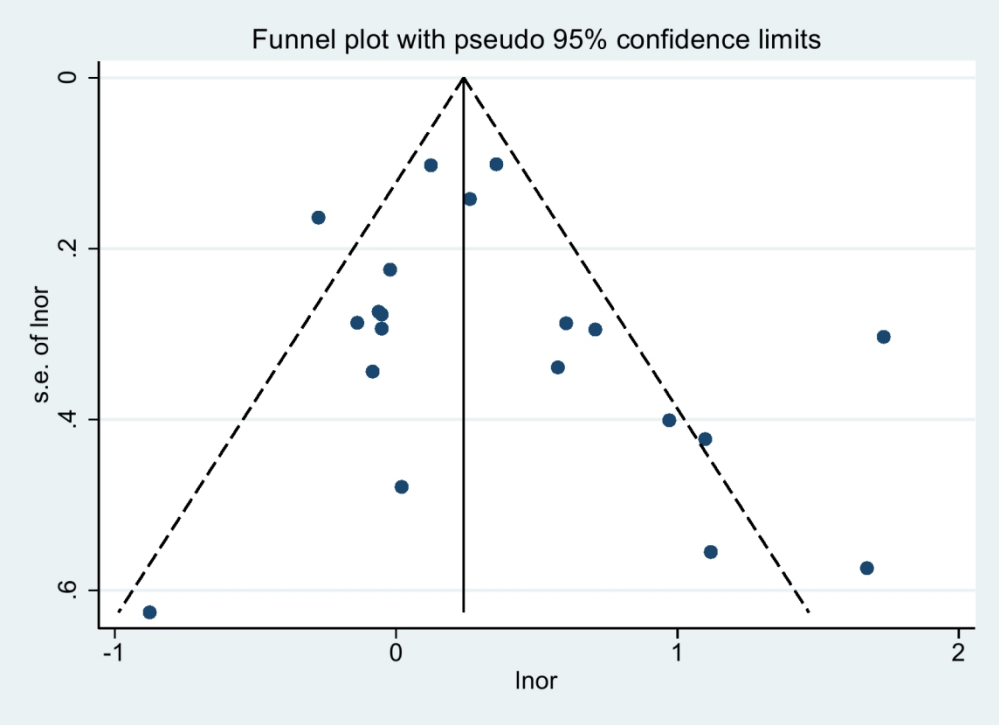

Fig 4

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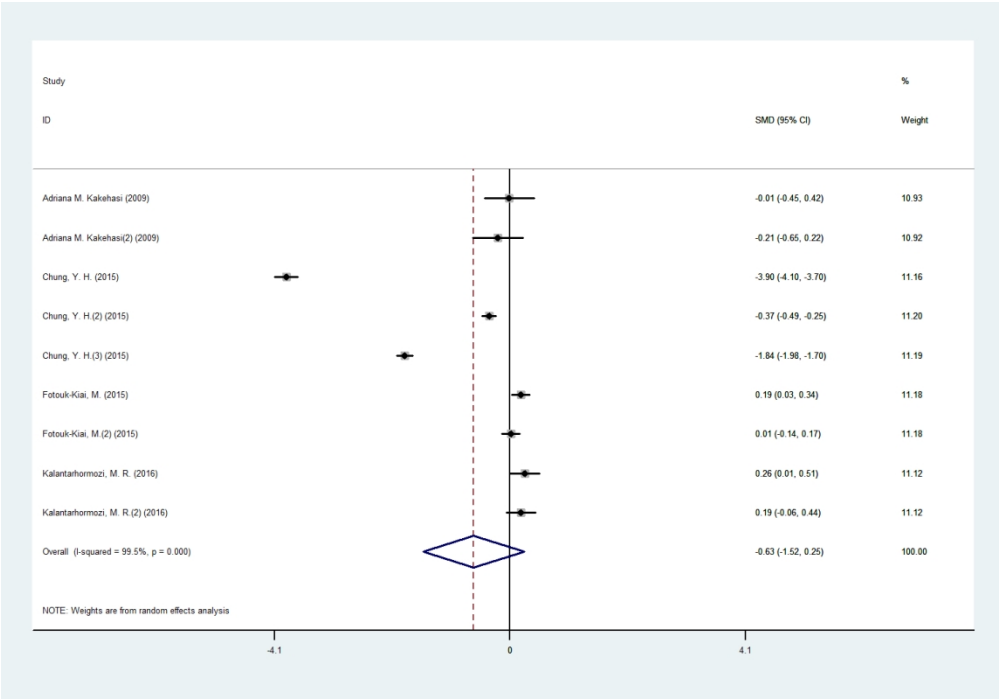

533x371mm (72 x 72 DPI)

PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	10
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	NA
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	14

BMJ Open

Relationship between Helicobacter pylori infection and osteoporosis: A systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-027356.R1
Article Type:	Research
Date Submitted by the Author:	04-Mar-2019
Complete List of Authors:	Wang, Taiwu; Third Military Medical University, Department of Epidemiology Li, Xiang; The Southwest Medical University, School of pharmacy Zhang, Qi; Center for Disease Control and Prevention of Eastern Theater Command Ge, Bingjie; 95810 Unit, The Chinese People's Liberation Army Zhang, Jinhai; Center for Disease Control and Prevention of Eastern Theater Command Yu, Lei; Third Military Medical University, Department of Epidemiology Cai, Tongjian; Third Military Medical University, Department of Epidemiology Zhang, Yao Xiong, Hongyan
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Infectious diseases, Epidemiology
Keywords:	osteoporosis, bone mineral density, Helicobacter pylori, meta-analysis

Original article

Relationship between *Helicobacter pylori* infection and osteoporosis: A systematic review and meta-analysis

Running Title: Relationship between *H. pylori* and osteoporosis

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Word count: 3311

Abstract

Objectives Many studies have explored the association between *Helicobacter pylori* (*H. pylori*) infection and osteoporosis. However, the results remain controversial. Therefore, we performed this systematic review and meta-analysis to evaluate the association between *H. pylori* infection and osteoporosis.

Design Systematic review and meta-analysis of case-control studies.

Data sources Databases, including PubMed, Embase, Web of Science and CBM, were screened from inception to April 30, 2018.

Eligibility Criteria Case-control studies aimed at assessing the association between *H. pylori* infection and osteoporosis.

Data extraction and analysis Study characteristics and study quality sections were reviewed. Studies were selected, and data were extracted by two reviewers. Pooled odds ratios (ORs) and 95% confidence intervals (95%CIs) were calculated using random effects model if heterogeneity existed, otherwise fixed effects model was used. Subgroup analyses were performed to explore the source of heterogeneity. Publication bias and sensitivity analyses were also tested.

Results A total of 21 studies with 9655 participants were included in our analyses. Taking together, we found that *H. pylori* infection was significantly associated with increased odds of osteoporosis (OR (95%CI): 1.39(1.13–1.71)); there was no significant difference between osteoporosis and osteopenia; the association between osteoporosis and *H. pylori* infection was relatively higher in males than females, but didn't reach significant level. However, the decrease of bone mineral density in *H.*

pylori positive patients was not significant when compared with *H. pylori* negative controls, which may due to the sample size.

Conclusions Our meta-analysis suggested that *H. pylori* infection was significantly associated with increased odds of osteoporosis. The clinicians should pay more attention to the patients infected with *H. pylori*. Further studies were still needed to exploring the confounding factors among studies and to elucidate the underlying biological mechanisms.

Keywords: osteoporosis; bone mineral density; *Helicobacter pylori*; meta-analysis.

Strengths and limitations of this study

- 21 studies with conflicting results were included for testing the association between osteoporosis and *Helicobacter pylori* infection.
- This is the third and most comprehensive meta-analysis, bringing the overall results of statistical significance and increased odds.
- From our results, the clinicians should pay more attention to the male patients infected with *H. pylori*.
- The results of the meta-analysis should be interpreted with caution due to the number and quality of studies included, and obvious heterogeneity.
- Causality can't be established in observational study.

Introduction

Helicobacter pylori (*H. pylori*), a Gram-negative and spiral-shaped bacterium dwelling on the gastric epithelium, has an influence on approximately 50% of the global population, especially those living in developing countries[1]. The prevalence of *H. pylori* infection is approximately 30% in developed countries and up to 80% in developing countries[2 3], and up to 90% in patients with dyspepsia[4]. In North Europe and North America, about one-third of adults are infected, and in South and East Europe, South America, and Asia, the prevalence of *H. pylori* is often higher than 50%[5]. Moreover, infected subjects born abroad (first-generation immigrants) had a higher risk of *H. pylori* infection than second-generation immigrants in a multi-ethnic European city[6]. *H. pylori* has been well-known to be associated with gastrointestinal diseases, such as gastritis, gastric ulcer, stomach cancer and so on[7]. Furthermore, some non-gastrointestinal diseases have also been proven to be associated with *H. pylori* by large-scale population researches or meta-analysis, such as preeclampsia[8], autoimmune thyroid diseases[9], myocardial infarction[10], hepatic encephalopathy[11] and prostatitis[12].

Osteoporosis is one of the most common metabolic bone diseases, characterized by decreased bone mineral density (BMD), increased bone fragility, and then increased susceptibility to fracture[13], especially in spine and hip. Osteoporosis has become a major health concern for both individuals and societies. Osteoporosis has huge adverse impacts on life quality and is associated with increased morbidity rates. The in-hospital mortality rate is between 0.85 to 2.26%[14]. In Europe, about half of women

and one-fifth of men aged over fifty years develop pathological fractures in hip, spine, forearm, or humerus due to osteoporosis during their remaining lifetime[15]. The same situation happens in other countries or districts, such as Japan and Taiwan[16 17].

There are well established evidence regarding the risk factors for osteoporosis [17], such as age, sex, body mass index (BMI), alcohol, and smoking. *H. pylori* infection can induce inflammatory and immune responses, such as increasing the level of IL-1 and TNF-α, which could trigger bone resorption, and regulate bone regeneration[18]. Recently, many studies about the association between osteoporosis and *H. pylori* have been performed. However, the role of *H. pylori* in osteoporosis remains controversial. This issue has been discussed in previous meta-analysis [19 20], but no significant association was found. As more studies evaluating the association between *H. pylori* infection and osteoporosis have been published since then [2 21-27], we carried out this updated meta-analysis to further evaluate the association between *H. pylori* infection and osteoporosis qualitatively, and the quantitative alterations of BMD in *H. pylori* infected patients compared with those in healthy controls.

Materials and Methods

This study was performed based on preferred reporting items for systematic reviews and meta-analyses (PRISMA)[28]. Study searching and selection, quality assessment, and data extraction were done by two researchers (TW and XL) independently to avoid bias, and disagreements were discussed by the two reviewers and by seeking the opinion of the third author (YZ) if necessary.

Search strategy

We searched through the databases of PubMed, Embase, Web of Science and Chinese Biomedical Literature Database (CBM). The last search for all databases was updated to April 30, 2018. We used the combined method of MeSH Term and free words by applying the following terms: *Helicobacter pylori*, *campylobacter pylori, H. pylori, hp, helicobacter, helicobacter bill, helicobacter hepaticus, helicobacter pullorum, helicobacter species, helicobacter sp, helicobacter genus, campylobacter, campylobacter infection, campylobacteriosis, Helicobacter pylori* infection, Helicobacter infection, *pylori, enterohepatic helicobacter spp, campylobacter sp* and fragility fracture, bone density, bone mass density, osteocalcin, bone loss, osteoporosis. Two authors evaluated potential publications by checking their titles and abstracts and then procured the most relevant publications for further examination. Bibliographies section of retrieved articles were also reviewed for additional pertinent studies which were possibly missed in the initial search.

Studies selection and data extraction

Studies were included if they met the following criteria: (1) it is an observational study; (2) its objective is to assess the association between *H. pylori* infection and osteoporosis, or compare the alteration of BMD between *H. pylori* positive and negative participants; (3) they either provided odds ratios (ORs) and 95% confidence intervals (95%CIs), or sufficient information was available to calculate the ORs and 95%CIs, or BMD in both *H. pylori* positive and negative participants. Articles were excluded if they were duplicate publications, reviews, animal studies, editorials, or

case reports. The papers were also excluded if no effect estimate was reported or not enough raw data for ORs and 95%CIs calculation was available. In the case of multiple studies with the same or overlapping data published by the same researchers, we selected the most recent study with the largest number of participants. All papers meeting the criteria defined above were included for further analysis.

The literatures included were carefully reviewed for information about the first author, publication year, country, population, sample size, sex, age, detection methods of *H. pylori* and osteoporosis, diagnosis location, diagnosis, and adjusted covariates.

If data could be acquired from the tabulated literature search results, they would be extracted carefully into 2x2 tables from all eligible publications by two independent reviewers. If data were not directly available, they would be calculated from published positive predictive values and/or negative predictive values if appropriate. The adjusted OR (95%CI), if existed, was adopted instead of rude OR (95%CI)[29]. In addition, for the studies comparing the BMD of participants with and without *H. pylori* infection, the data on BMD was also extracted.

Quality assessment

Quality assessment was performed using the Newcastle-Ottawa quality assessment scale(NOS)[30]. Two researchers conducted blinded quality assessment of the included literatures. The NOS assigns a maximum of 9 points to studies of highest quality according to three quality parameters: selection, comparability, and outcome.

Statistical analyses

The primary measures were ORs and 95%CIs for the association between *H. pylori*

infection and osteoporosis, and standardized mean difference (SMD) for BMD alterations between *H. pylori* positive and negative participants. To assess heterogeneity among the studies, we calculated the Cochran's Chi-squared test (with $P < 0.10$ indicating statistically significant heterogeneity) and the statistic I^2 (The heterogeneity might not be important with I^2 of 0 to 40%, while moderate heterogeneity with I^2 of 30 to 60%, substantial heterogeneity with I^2 of 50 to 90% and considerable heterogeneity with I^2 of 75 to 100%)[31]. The pooled results were calculated using fixed effects model (Inverse Variance) if no obvious heterogeneity existed, otherwise random effects model (I-V heterogeneity) was used ($P < 0.10$ was considered indicative of obvious heterogeneity). The cumulative meta-analysis was conducted for the extracted data using a pooled random effects model with the publication year. In the event of obvious heterogeneity, subgroup analysis was performed according to sex, postmenopausal or not, country, Asian or not, detection methods of *H. pylori*, detection methods of osteoporosis, and detection location of dual-energy X-ray absorptiometry (DEXA). Meta regression (using ReML methods) was also performed to explore the potential heterogeneity. Publication bias was assessed by funnel plot and Egger's test[32]. A sensitivity analysis was completed by converting the pooled results from random effects model into fixed effects model or from fixed effects model into random effects model. All statistical analyses were performed using Stata 12.0.

Patient and public involvement

There was no patient and public involvement as this was a database research study.

Results

Search results

Using our search strategy, a total of 1720 articles were identified through PubMed, Web of Science, Embase and CBM, and one additional record was identified through other sources. Then, 443 duplicate papers were removed firstly, and 1242 papers were excluded after scanning their titles and abstracts. After screening the full texts of the included articles, 15 studies were excluded for the following reasons: duplicate publication(n=4), not about the topic(n=2), no related data about *H. pylori* and/or osteoporosis(n=4), lack of access to full text (n=5). A total of 21 studies [2 16 21-27 33-44] were included for further analysis (Figure 1).

Study Characteristics

A total of 21 articles were included in this study. Of the 21 articles included, 20 provided data for association between *H. pylori* and osteoporosis[2 16 21-27 33-41 43 44], 4 for the BMD alterations in *H. pylori* positive participants compared with negative controls[23 34 35 42], 3 provided both[23 34 35]. All these studies were published from 2005 to 2018. Four studies were conducted in China, 3 in Iran, 1 in Italy, 9 in Japan, 2 in Brazil, 1 in Korea and 1 in Turkey. As to the sex of participants, 4 were postmenopausal women, 4 were females, 4 were males, 9 involved both males and females. The detection methods of *H. pylori* were mainly ELISA and ^{13}C-urea breath test, while the detection methods of osteoporosis were DEXA and quantitative ultrasound. As to the diagnosis, 5 were osteopenia, 13 were osteoporosis, and 2 provided decreased BMD (treated as osteopenia for analysis) (Table 1). In addition,

12 studies showed no significant associations of *H. pylori* infection and osteoporosis (or osteopenia), while 8 showed significant associations.

Quality evaluation

The Newcastle-Ottawa scale (NOS) was adopted to evaluate the quality of these case-control studies. Among the selection items, the evaluation results ranged from 4 to 8, with the median NOS score was 6, indicating a medium quality of the studies included. The most common source of bias came from selection and comparability. (Table 1)

Synthesis of the results

As shown in Figure 2, the overall OR was obtained based on the 20 studies involving the *H. pylori* and osteoporosis (including osteopenia) (a total of 8788 patients and healthy controls). As the existence of obvious heterogeneity (Chi-square = 69.60, I^2 = 72.7%, $P < 0.01$), random effect model was used and the pooled results of OR and its 95%CI were 1.37(1.11,1.69), indicating *H. pylori* infection was significantly associated with increased odds of osteoporosis/osteopenia. A cumulative meta-analysis was conducted with publication year in ascending order, and the results indicated that the pooled OR (95% CI) started to show statistical significance at 1.57 (95% CI: 1.02,2.41) from the ninth analyzed study, with gradually stabilizing results afterwards (Figure 3).

Subgroup analyses

Given that obvious heterogeneity existed, subgroup analyses were performed based on the potential confounding factors. All 20 studies were involved in these subgroup

analyses. Figure 4 showed that both osteoporosis and osteopenia were significantly associated with *H. pylori* infection with OR(95%CI) of 1.61(1.11, 2.32) and 1.22(1.07, 1.39) respectively. Although the OR was a little higher in osteoporosis group, the meta regression analysis showed no significant difference between these two groups (t=1.18, *P*=0.26). Therefore, we pooled osteoporosis and osteopenia together to analyze other confounding factors.

Results of subgroup analyses by other factors were shown in Table 2. We found that the association between *H. pylori* infection and osteoporosis was significant in males but not in females. However, meta regression analysis showed no significant difference between these two groups. Moreover, no significant associations between *H. pylori* infection and osteoporosis were observed in either the postmenopausal women or non-postmenopausal women subgroup. When stratified by countries, we found significant associations between *H. pylori* infection and osteoporosis in China, Japan, and Korea (three East Asian countries). Other factors that may affect the results were presented in Table 2.

Publication bias and sensitivity analyses

Funnel plot was used to examine the results of this meta-analysis. As shown in Figure 5, the funnel plot indicated no publication bias, which was also confirmed by Egger's test (t = 1.57, *P* = 0.13) (Figure 6). Sensitivity analysis was also performed by converting the pooled model from the random effects model to the fixed effects model. The result of fixed effects model was 1.21(1.10-1.33), showed no obvious differences compared with the result of random effects model, indicating the pooled results was

relatively stable.

Alterations of BMD in *H. pylori* infected population

Four studies were involved in this meta-analysis[23 34 35 42]. As each study has two different detection locations, we performed subgroup analysis based on detection locations. As shown in supplementary Figure 1, the BMD(g/cm^2) alterations between *H. pylori* positive and negative participants were -0.01(-0.45,0.42) for hip, -0.94(-3.15,1.28) for lumber and -0.04(-0.40,0.31) for femur using random effects model as obvious heterogeneity existed. No significant associations were observed so far.

Discussion

Although osteoporosis isn't a deadly disease, it causes huge burden to individuals and society owing to its high morbidity. Here, we got a comprehensive result by meta-analysis, indicating that *H. pylori* infection may be a risk factor for osteoporosis. However, the mechanism is still unclear. Several possible mechanisms may explain this result. First, *H. pylori* infection may lead to systemic inflammation, and release of cytokines, such as tumor necrosis factor-alpha, interleukin-1 and interleukin-6[45], which may cause bone turnover indirectly. Second, many studies have shown that low vitamin B12 may be associated with *H. pylori* infection[46]. If the serum vitamin B12 levels are decreased, the folate becomes trapped as methyltetrahydrofolate and interrupts for folate-related DNA synthesis, which is an important factor for bone remodeling. Therefore, the decrease of vitamin B12 may lead to decreased BMD and

osteoporosis[47]. Third, *H. pylori* infection may decrease the calcium absorption by causing the gastric mucosal atrophy and decreasing acid secretion. Thus, eradication of *H. pylori* may increase calcium absorption and stop the process of osteoporosis through decreasing the levels of inflammatory cytokines and improving gastric mucosal atrophy.

The present meta-analysis of 20 studies indicated that patients with *H. pylori* infection were associated with an estimated 1.37 times higher odds ratios of developing osteoporosis as compared with those without *H. pylori* infection, while no associations were found in previous meta-analysis [19 20] (one had 5 studies involving 1321 participants, and one had 4 studies involving 520 participants). As the previous meta-analysis studies had no quality assessment and our analysis included more studies and participants, the results in our study might be more reliable than the previous meta-analysis studies.

Despite the significant association between *H. pylori* infection and osteoporosis, obvious heterogeneity existed between the included studies. We found that sex of participates may affect the results. As known to all, female and pausimenia women are independent risk factors of osteoporosis. Here, we explored the relationship between osteoporosis and *H. pylori* infection, and found that the relationship was significant in male, but not in female (whether pausimenia or not), which wasn't paradoxical with the fact that women with postmenopausal should have a higher risk of osteoporosis than men. In the group of both sexes, the results showed statistic difference and obvious heterogeneity, which may be due to the ratio of M/F and other

confounding factors. Therefore, we might suggest that more attention should be paid to male than female in *H. pylori* positive patients. However, only 7 studies (4 were about postmenopausal women and 3 were about non-postmenopausal women) were conducted in female, the results may be not that reliable due to the small sample size. Another reason may also be possible, that the different degree of osteoporosis may affect the diagnosis and some early patients may be regarded as healthy controls. Further studies with dose-response relationship of different severity of osteoporosis and prevalence may help to confirm this hypothesis. In the subgroup analysis by criteria (osteoporosis and osteopenia), the OR in osteoporosis was a little higher than that in osteopenia, which may also help to prove our hypothesis. In the subgroup analysis based on countries, significant association was evidenced in three East-Asian countries (China, Japan, Korea), indicating many other factors that were associated with geography may affect the results.

In our research, we also explored the heterogeneity from diagnosis methods factors. We found that the detection methods of osteoporosis (DEXA and quantitative ultrasound) affected the pooled results, and the detection locations of DEXA also contributed to the heterogeneity. From our results, we thought that DEXA might be a better tool to diagnose osteoporosis in assessing the association between *H. pylori* and osteoporosis. The same situation also happened in the detection methods of *H. pylori*. We found ELISA and multi-method strategy may provide more homogeneous results. In total, in despite of the significant association between *H. pylori* and osteoporosis, as the heterogeneity still existed obviously, further studies were still needed to address

its potential confounding factors.

In a previous meta-analysis study, Karn Wijarnpreecha et al found increased odds of nonalcoholic fatty liver disease (NAFLD) among patients infected with *H. pylori* [48]. However, Sikarin Upala et al found that no significant difference in BMD between patients with fatty liver disease and controls[49]. Combine the two meta-analysis and our results, we may guess that *H. pylori* may be an independent risk factor of NAFLD and osteoporosis, and/or *H. pylori* infection may be an important confounding factor in exploring the relationship between NAFLD and osteoporosis, or no actual relationship between NAFLD and osteoporosis exists. However, as the authors stated[49], the review was a preliminary result because of limited amount of literature, it might be too early to have definite conclusion.

We also compared the quantitative alterations of BMD in *H. pylori* infected subjects. However, no significant difference was found. The reason may be that: 1) the sample size was relatively small, 2) the severities of osteoporosis were not serious, or the infection of *H. pylori* didn't last long enough to cause alterations, 3) though the basic characteristics of included studies were comparable, many other confounding factors that might affect BMD have not been adjusted. Therefore, more studies with large sample size were still needed to verify the alterations of BMD in *H. pylori* infection.

The strength of the present meta-analysis lies in inclusion of 21 observational studies reporting data on *H. pylori* infection and osteoporosis, and the alterations of BMD by *H. pylori*. However, our meta-analysis has several limitations that should be recognized when interpreting the results. First, most of the included studies were

hospital-based or health center-based, which were not affected by detection bias, but might be subjected to selection bias. However, the prevalence of *H. pylori* infection in most studies that we selected was consistent with the incidence rate in the general population. Second, our analysis had an ascertainment bias that might be present because progression of osteoporosis is continuous, and some patients may be classified as controls. However, this may lead to a more conservative result, which may help to indicate that our overall result is reliable. Third, the heterogeneity is still obvious. However, we performed subgroup analyses based on study characteristics, and found that some factors may affect the association. In addition, when available, adjusted estimates were used in preference to unadjusted estimates. Even though the adjusted estimates may be closer to the true effect, the different adjusted factors in different studies may also contribute to the heterogeneity. Four, the qualities of included studies were medium, and some studies were published informally. We also included all these studies based on inclusion and exclusion criteria to avoid publication bias. Nevertheless, our study is still the most comprehensive about the association between *H. pylori* infection and osteoporosis.

In summary, our results suggest significant increased odds of osteoporosis in patients with *H. pylori* infection. The clinicians should pay more attentions to the patients infected with *H. pylori* by using DEXA scan, especially those chronic gastritis patients. However, the results should be cautiously interpreted considering the heterogeneity and the fact that all studies are non-randomized and retrospective. Further studies are needed to explore the mechanism and confounding factors

between *H. pylori* and osteoporosis.

Funding

This work was supported by the National Natural Science Foundation of China [No. 81202220 to Y. Z.; No. 81673211 and 81372952 to T. C.], by the Natural Science Foundation of Jiangsu Province, China [No. BK20150094 to Q.Z.], and by the Chongqing Health and Family Planning Commission [No. 20141027 to H. X.].

Contributors

YZ and HX led the study by designing, interpreting results, and revising manuscript critically for important intellectual content; TW and XL contributed to data analysis, result interpretation and drafting of the manuscript; QZ, BG and JZ participated in study data collection and revising manuscript; TW, XL, TC and LY participated in study conduct and results interpretation. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Patient consent Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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Figure legend

Figure 1. Flow diagram of the article selection for systematic review.

Figure 2. Forest plot of the included studies assessing the association between *Helicobacter pylori* and osteoporosis (random effects models). CI indicates confidence interval.

Figure 3. Forest plot of cumulative meta-analysis.

Figure 4. Forest plot of subgroup meta-analysis according to diagnosis (random effects models).

Figure 5. Funnel plot of publication bias for the association between *Helicobacter pylori* and osteoporosis.

Figure 6. The Egger's test for publication bias.

Supplementary figure 1. Meta-analysis of SMD according to detection locations.

Table 1. Characteristics and quality assessment of the studies included

Author [Reference]	Year	Country	Sex(M/F)	Age(mean age±SD or (range age) years)	Detection method of *H.pylori*	Detection methods of osteoporosis	Diagnosis locations	Diagnosis	Cases/Controls/ Total	Scores of NOS	Main adjusted factors (the methods used for adjusting)
Figura, N.[44]	2005	Italy	Males	65 (55–82) for patients; 64.5(55–80) for controls	ELISA	DEXA	Lumbar and femur bone	Osteoporosis	80/160/240	7	age, socioeconomic background, and smoking habits.
Adriana M. KAKEHASI[43]	2007	Brazil	Postmenopausal women	61.6±7(50–79)	non-ELISA	DEXA	Lumbar spine	Osteoporosis	18/32/50	6	mean age, body mass index, age at menarche, postmenopausal period
Adriana M. Kakehasi[42,#]	2009	Brazil	Postmenopausal women	63.7±7.3 for Hp(+) 62.5±7.0 for Hp(-)	non-ELISA	DEXA	Lumbar spine and hip	Not fitted*	34/27/61	-	age, Postmenopausal time, BMI
Akkaya, Nuray[41]	2011	Turkey	Postmenopausal women	65.29±6.09 patients; 63.57±6.53 controls	ELISA	DEXA	Lumbar and femur neck	Osteoporosis	58/47/105	6	age, education level, occupation, age of menarche or menopause, duration of postmenopausal, period or daily consumption of tea, coffee, alcohol or dairy products
Chinda, D.[40]	2013	Japan	379/631	not mentioned	ELISA	QU	Calcaneal osteo	Osteopenia	-/-/1010	7	age, BMI, smoking, alcohol consumption, periodical exercise, latest educational level (logistic regression analysis)
Asaoka, Daisuke[39]	2014	Japan	95/105	63.1±8.8 years	Both	DEXA	Lumbar vertebrae	Osteoporosis	41/159/200	6	age, gender, BMI, alcohol consumption, smoking, BAP, PUD, and EGA (multivariate logistic regression analysis)
Asaoka, D.[38]	2014	Japan	131/26	71.1±7.5 patients 61.6±8.9 controls	not mentioned	DEXA	Lumbar	Osteoporosis	24/133/157	6	age, sex, BMI, Brinkman Index (B.I.), accmulated amount of alcohol (multivariate analysis)
Lin, S. C.[37]	2014	China	Female	77 (65–97)	non-ELISA	DEXA	Not mentioned	Osteoporosis	101/264/365	5	age group, body mass index group, and use of proton pump inhibitor (multivariate logistic regression analyses)
Asaoka, D.[36]	2015	Japan	130/134	69.8±6.8 for patients 61.9±8.2 for controls	not mentioned	DEXA	Not mentioned	Osteoporosis	45/219/264	7	age, sex, BMI. etc (multivariate analysis)
Asaoka, D.[16]	2015	Japan	120/135	63.2±8.5	Both	DEXA	Lumbar vertebrae	Osteoporosis	43/212/255	6	age, sex, BMI, cumulative alcohol intake, Brinkman index, type 2 diabetes mellitus, calcium channel blocker, PPI, hemoglobin, calcium, gamma glutamyl transpeptidase, bone-specifi alkaline phosphatase, NTX, hiatal hernia, and EGA (multivariate logistic regression analysis)
Chung, Y. H.[35,#]	2015	Korea	Men	54.4±10.7 for Hp+ 51.9±12.1 for Hp-	ELISA	DEXA	Lumbar (L1–L4)	osteopenia	-/-/1126	7	Height, weight, BMI, alcohol, exercise.
Fotouk-Kiai, M.[34,#]	2015	Iran	575/392	68.3±6.8 for hp+ 69.3±7.4 for hp-	ELISA	DEXA	Lumbar vertebra	Osteoporosis	314/653/967	5	age, sex, smoking, alcohol consumption and BMI

							and Femur				
Mizuno, S.[33]	2015	Japanese	Men	62.1±5.0 for low TBD 58.4±5.7 for normal	ELISA	QU	Not mentioned	Decreased BMD	116/114/230	8	Age, BMI and smoking habit (logistic regression analysis)
Chinda, D.[21]	2016	Japan	Men	50.2±15.4 years	ELISA	QU	Not mentioned	Osteopenia	-/-/295	7	age, BMI, serum level of estradiol, the intake of calcium per day, smoking, drinking, periodical exercise, last educational background (logistic regression)
Chinda, D.[22]	2016	Japan	Females	52.2±15.2	ELISA	QU	Not mentioned	Osteopenia	-/-/473	6	age, BMI, smoking, alcohol consumption, periodical exercise, last educational level, serum level of estradiol, calcium intake per day (multiple logistic regression)
Kalantarhormozi, M. R.[23,#]	2016	Iran	Postmenopausal women	58.87±8.02	ELISA	DEXA	lumbar spine and femur	Osteoporosis	16/234/250	6	age and BMI (multiple linear regression)
Zhang, Chaoxian[24]	2016	China	194/126	38.32±10.64 for patients 38.27±7.46 for controls	non-ELISA	DEXA	Not mentioned	Osteoporosis	160/160/320	5	Age, gender, gene (multiple logistic regression)
Abdolahi, N.[25]	2017	Iran	Postmenopausal women	not mentioned	ELISA	not mentioned	Not mentioned	Osteoporosis	73/34/107	8	not mentioned
Chinda, D.[26]	2017	Japan	Females	62.5±8.6 for patients 44.9±10.9 for controls	ELISA	QU	Calcaneus	Osteopenia	197/276/473	4	age, smoking and drinking habit, schooling duration, estradiol levels, menopause, birth history (multiple logistic regression analysis)
Lu, Li-juan[2]	2018	China	1474/393	54.0±9.6	non-ELISA	QU	Calcaneus	Osteoporosis	900/967/1867	6	gender and age
Pan, B. L.[27]	2018	China	568/299	55.9±11.3	non-ELISA	DEXA	Not mentioned	Decreased BMD	311/556/867	5	Sex, age, BMI, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and peptic ulcer disease (multiple stepwise logistic regression analyses)

Not fitted *: this study only explored the alteration of BMD in patients with *H. pylori* infection.

#: this study also reported the BMD in patients with *H. pylori* infection.

ELISA: enzyme linked immunosorbent assay; QU: quantitative ultrasound; DEXA: dual energy X-ray absorptiometry scan; NOS：Newcastle-Ottawa scale

Table 2. Overall effect estimates for *Helicobacter pylori* infection and osteoporosis according to study characteristics

Factors	Categories	No. of studies	OR [95%CI]	Model used	Heterogeneity		Meta-regression	
					I^2	*P*-value	t	*P*-value
Sex*								
	Female	8	1.09(0.87,1.35)	Fixed	33.0%	0.17	-	-
	Male	5	1.27(1.07,1.50)	Fixed	14.6%	0.32	0.47	0.64
	Both	9	1.21(1.07,1.37)	Random	85.6%	0.00	1.78	0.09
Postmenopausal or not								
	Non-postmenopausal women	4	1.08 (0.83,1.41)	Fixed	48.0%	0.12	-	-
	Postmenopausal women	4	1.09(0.75,1.58)	Fixed	35.8%	0.20	-0.13	0.90
Country								
	China	4	1.86(1.06,3.28)	Random	90.4%	0.00	-	-
	Japan	9	1.57(1.08,2.28)	Random	63.7%	0.005	-0.39	0.70
	Italy	1	0.87(0.50,1.53)		-	-	-1.11	0.29
	Brazil	1	0.42(0.12,1.42)		-	-	-1.69	0.11
	Korea	1	1.29(1.05,1.57)		-	-	-0.59	0.57
	Iran	3	1.06(0.60,1.86)	Random	61.3%	0.075	-1.16	0.27
	Turkey	1	0.95(0.53,1.69)		-	-	-0.98	0.34
Asian country or not								
	Non-Asian country	2	0.77(0.46,1.28)	Fixed	12.8%	0.28	-	-
	Asian country	18	1.44(1.16,1.79)	Random	73.9%	0.00	1.60	0.13
Detection methods of *H. pylori*								
	ELISA	11	1.09(0.96,1.24)	Fixed	32.1%	0.14	-	-
	Non-ELISA	5	1.62(0.96,2.72)	Random	88.4%	0.00	1.52	0.15
	Both	2	3.67(1.88, 7.16)	Fixed	0%	0.42	2.65	0.02
Detection methods of osteoporosis								
	DEXA	13	1.58(1.14,2.18)	Random	79.5%	0.00	-	-
	QU	6	1.05(0.90,1.22)	Fixed	0%	0.51	-1.33	0.20
Detection location of DEXA								

	Lumbar	6	1.75(0.99,3.07)	Random	65.4%	0.013	-	-
	Femur	3	1.56(1.17,2.08)	Fixed	0%	0.90	-0.17	0.87

*: One study has both males, females and over results, it was used three times in subgroups analysis (males, females, and both).

ELISA: enzyme linked immunosorbent assay; QU: quantitative ultrasound; DEXA: dual energy X-ray absorptiometry scan.

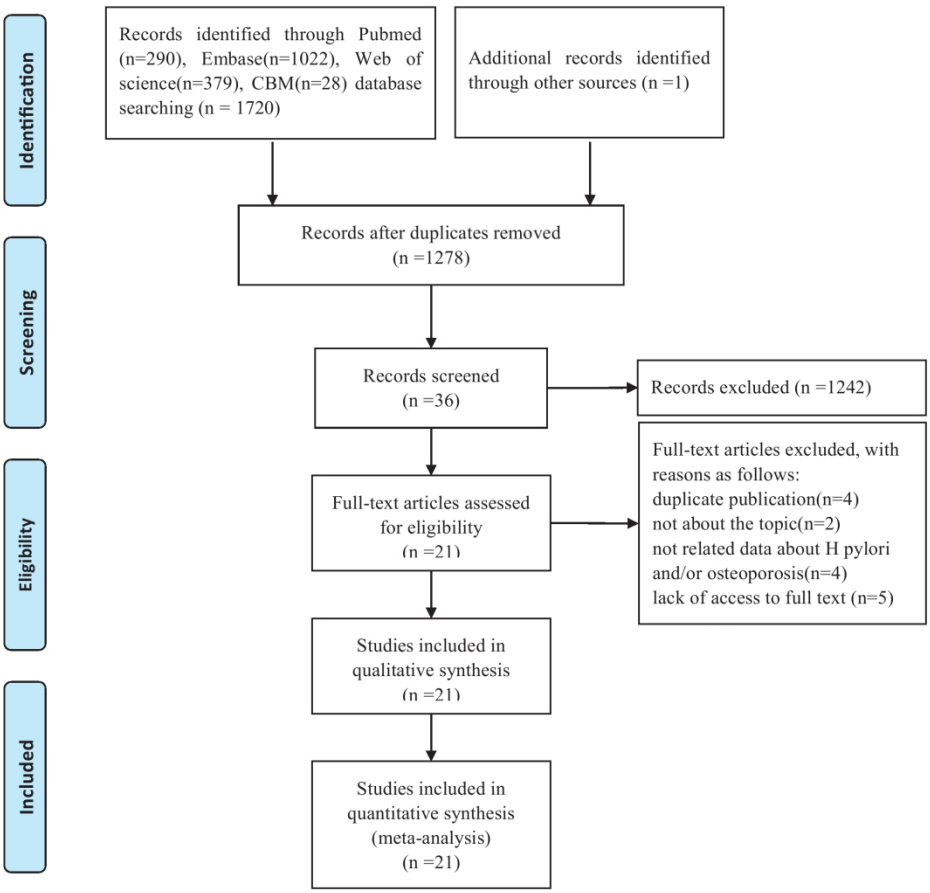

Fig 1

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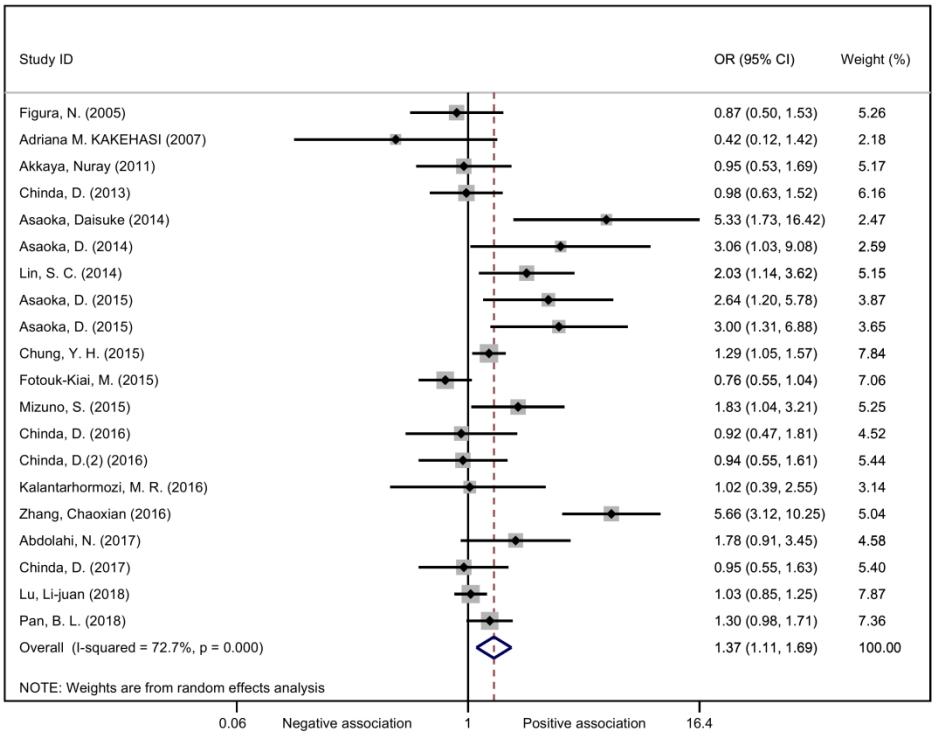

Fig 2

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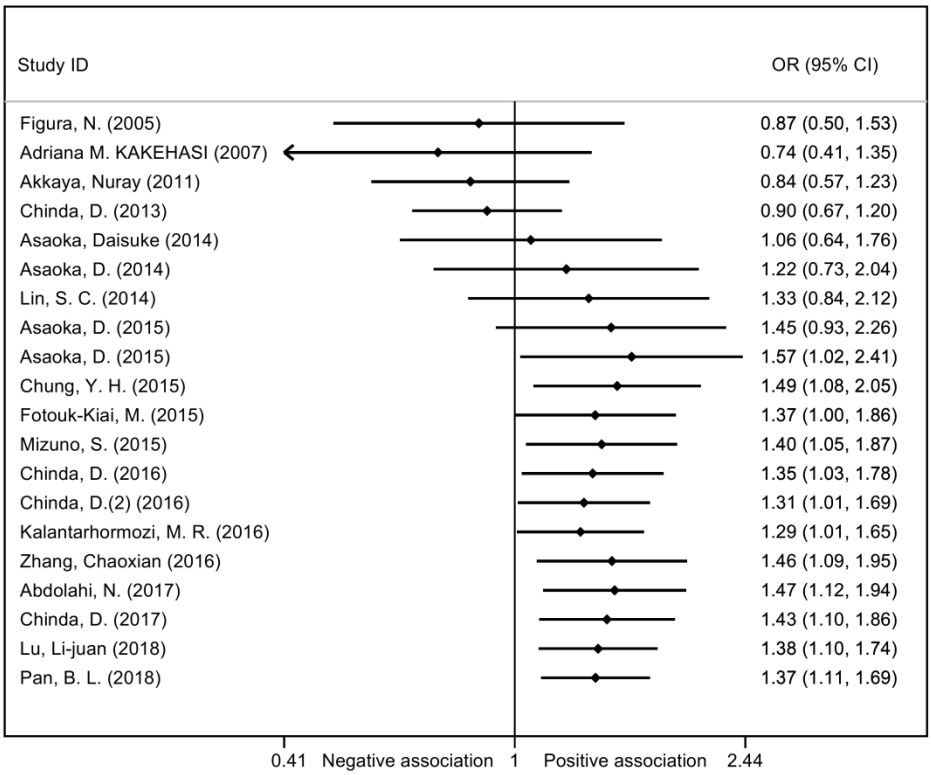

Fig 3

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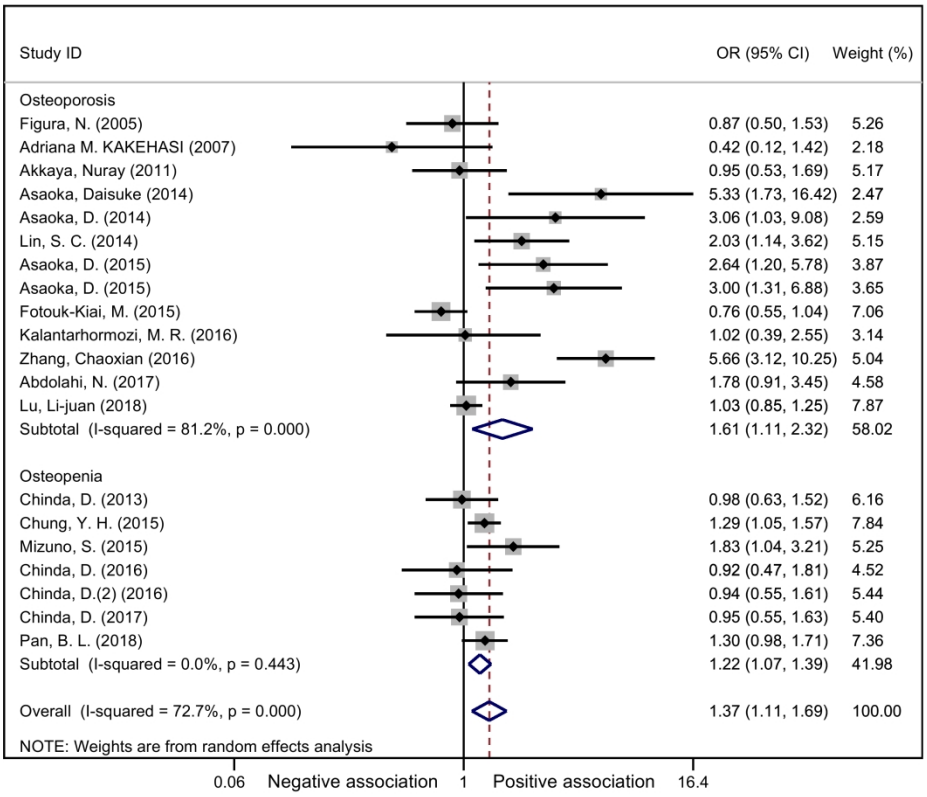

Fig 4

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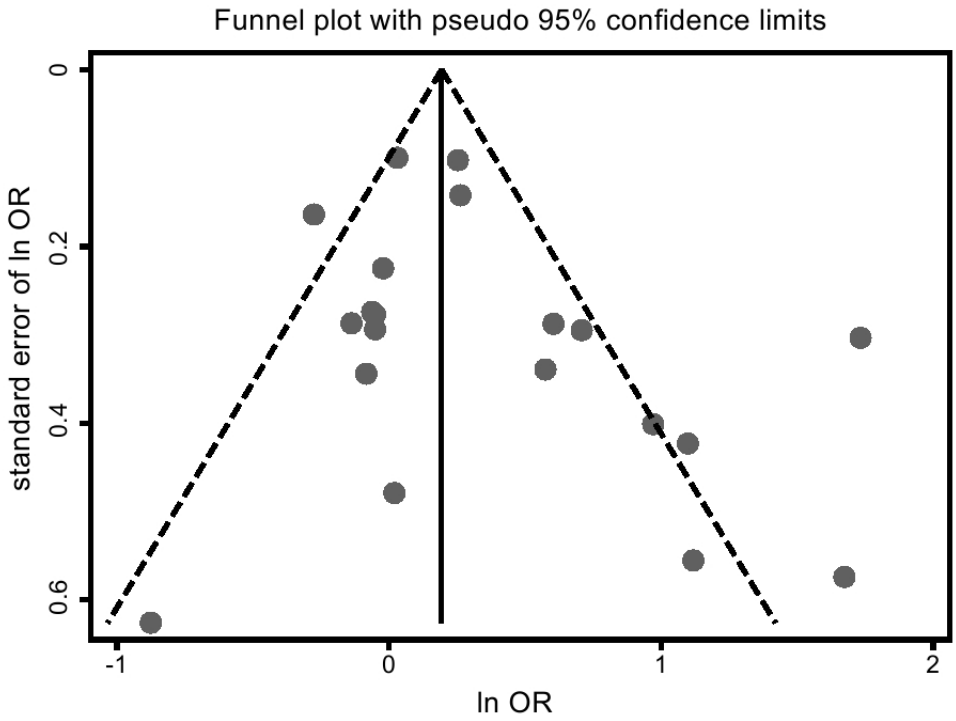

Fig 5

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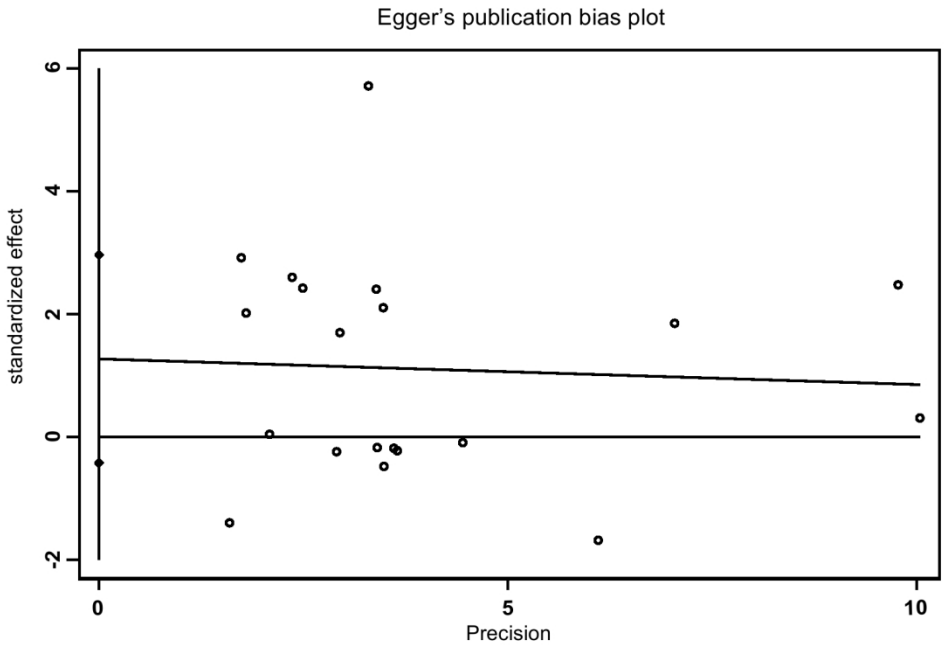

Fig 6

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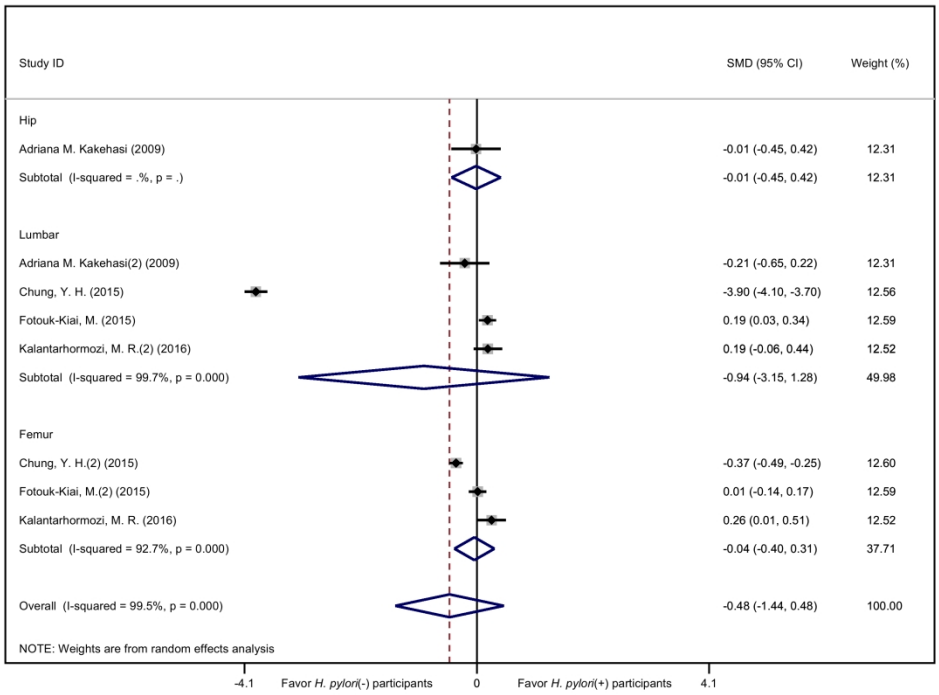

277x215mm (300 x 300 DPI)

PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	10
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	NA
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	12
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	16
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	17

BMJ Open

Relationship between Helicobacter pylori infection and osteoporosis: A systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-027356.R2
Article Type:	Research
Date Submitted by the Author:	24-Apr-2019
Complete List of Authors:	Wang, Taiwu; Third Military Medical University, Department of Epidemiology Li, Xiang; The Southwest Medical University, School of pharmacy Zhang, Qi; Center for Disease Control and Prevention of Eastern Theater Command Ge, Bingjie; 95810 Unit, The Chinese People's Liberation Army Zhang, Jinhai; Center for Disease Control and Prevention of Eastern Theater Command Yu, Lei; Third Military Medical University, Department of Epidemiology Cai, Tongjian; Third Military Medical University, Department of Epidemiology Zhang, Yao Xiong, Hongyan
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Infectious diseases, Epidemiology
Keywords:	osteoporosis, bone mineral density, Helicobacter pylori, meta-analysis

Original article

Relationship between *Helicobacter pylori* infection and osteoporosis: A systematic review and meta-analysis

Running Title: Relationship between *H. pylori* and osteoporosis

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Word count: 3328

Abstract

Objectives Many studies have explored the association between *Helicobacter pylori* (*H. pylori*) infection and osteoporosis. However, the results remain controversial. Therefore, we performed this systematic review and meta-analysis to evaluate the association between *H. pylori* infection and osteoporosis.

Design Systematic review and meta-analysis of case-control studies.

Data sources Databases, including PubMed, Embase, Web of Science and CBM, were screened from inception to April 30, 2018.

Eligibility Criteria Case-control studies aimed at assessing the association between *H. pylori* infection and osteoporosis.

Data extraction and analysis Study characteristics and study quality sections were reviewed. Studies were selected, and data were extracted by two reviewers. Pooled odds ratios (ORs) and 95% confidence intervals (95%CIs) were calculated using random effects model if heterogeneity existed, otherwise fixed effects model was used. Subgroup analyses were performed to explore the source of heterogeneity. Publication bias and sensitivity analyses were also tested.

Results A total of 21 studies with 9655 participants were included in our analyses. Taking together, we found that *H. pylori* infection was associated with increased odds of osteoporosis (OR (95%CI): 1.39(1.13–1.71)); there was no significant difference between osteoporosis and osteopenia; the association between osteoporosis and *H. pylori* infection was relatively higher in males than females, but didn't reach significant level. However, the decrease of bone mineral density in *H. pylori* positive

patients was not significant when compared with *H. pylori* negative controls, which may due to the sample size.

Conclusions Our meta-analysis suggests an association between osteoporosis and Helicobacter pylori infection. The clinicians should pay more attention to the patients infected with *H. pylori*. Further studies were still needed to exploring the confounding factors among studies and to elucidate the underlying biological mechanisms.

Keywords: osteoporosis; bone mineral density; *Helicobacter pylori*; meta-analysis.

Strengths and limitations of this study

- 21 studies with conflicting results were included for testing the association between osteoporosis and *Helicobacter pylori* infection.
- This is the third and most comprehensive meta-analysis, bringing the overall results of statistical significance and increased odds.
- The results of the meta-analysis should be interpreted with caution due to the number and quality of studies included, and obvious heterogeneity.
- Causality can't be established in observational study as the chronological order between *Helicobacter pylori* infection and osteoporosis can't be confirmed.

Introduction

Helicobacter pylori (*H. pylori*), a Gram-negative and spiral-shaped bacterium dwelling on the gastric epithelium, has an influence on approximately 50% of the global population, especially those living in developing countries[1]. The prevalence of *H. pylori* infection is approximately 30% in developed countries and up to 80% in developing countries[2 3], and up to 90% in patients with dyspepsia[4]. In North Europe and North America, about one-third of adults are infected, and in South and East Europe, South America, and Asia, the prevalence of *H. pylori* is often higher than 50%[5]. Moreover, infected subjects born abroad (first-generation immigrants) had a higher risk of *H. pylori* infection than second-generation immigrants in a multi-ethnic European city[6]. *H. pylori* has been well-known to be associated with gastrointestinal diseases, such as gastritis, gastric ulcer, stomach cancer and so on[7]. Furthermore, some non-gastrointestinal diseases have also been proven to be associated with *H. pylori* by large-scale population researches or meta-analysis, such as preeclampsia[8], autoimmune thyroid diseases[9], myocardial infarction[10], hepatic encephalopathy[11] and prostatitis[12].

Osteoporosis is one of the most common metabolic bone diseases, characterized by decreased bone mineral density (BMD), increased bone fragility, and then increased susceptibility to fracture[13], especially in spine and hip. Osteoporosis has become a major health concern for both individuals and societies. Osteoporosis has huge adverse impacts on life quality and is associated with increased morbidity rates. The in-hospital mortality rate is between 0.85 to 2.26%[14]. In Europe, about half of women

and one-fifth of men aged over fifty years develop pathological fractures in hip, spine, forearm, or humerus due to osteoporosis during their remaining lifetime[15]. The same situation happens in other countries or districts, such as Japan and Taiwan[16 17].

There are well established evidence regarding the risk factors for osteoporosis [17], such as age, sex, body mass index (BMI), alcohol, and smoking. *H. pylori* infection can induce inflammatory and immune responses, such as increasing the level of IL-1 and TNF-α, which could trigger bone resorption, and regulate bone regeneration[18]. Recently, many studies about the association between osteoporosis and *H. pylori* have been performed. However, the role of *H. pylori* in osteoporosis remains controversial. This issue has been discussed in previous meta-analysis [19 20], but no significant association was found. As more studies evaluating the association between *H. pylori* infection and osteoporosis have been published since then [2 21-27], we carried out this updated meta-analysis to further evaluate the association between *H. pylori* infection and osteoporosis qualitatively, and the quantitative alterations of BMD in *H. pylori* infected patients compared with those in healthy controls.

Materials and Methods

This study was reported based on preferred reporting items for systematic reviews and meta-analyses (PRISMA)[28]. Study searching and selection, quality assessment, and data extraction were done by two researchers (TW and XL) independently to avoid bias, and disagreements were discussed by the two reviewers and by seeking the opinion of the third author (YZ) if necessary.

Search strategy

We searched through the databases of PubMed, Embase, Web of Science and Chinese Biomedical Literature Database (CBM). The last search for all databases was updated to April 30, 2018. We used the combined method of MeSH Term and free words by applying the following terms: *Helicobacter pylori*, *campylobacter pylori, H. pylori, hp, helicobacter, helicobacter bill, helicobacter hepaticus, helicobacter pullorum, helicobacter species, helicobacter sp, helicobacter genus, campylobacter, campylobacter infection, campylobacteriosis, Helicobacter pylori* infection, Helicobacter infection, *pylori, enterohepatic helicobacter spp, campylobacter sp* and fragility fracture, bone density, bone mass density, osteocalcin, bone loss, osteoporosis. The search strategy is presented in the Appendix S1. Two authors evaluated potential publications by checking their titles and abstracts and then procured the most relevant publications for further examination. Bibliographies section of retrieved articles were also reviewed for additional pertinent studies which were possibly missed in the initial search.

Studies selection and data extraction

Studies were included if they met the following criteria: (1) it is an observational study; (2) its objective is to assess the association between *H. pylori* infection and osteoporosis, or compare the alteration of BMD between *H. pylori* positive and negative participants; (3) they either provided odds ratios (ORs) and 95% confidence intervals (95%CIs), or sufficient information was available to calculate the ORs and 95%CIs, or BMD in both *H. pylori* positive and negative participants. Articles were

excluded if they were duplicate publications, reviews, animal studies, editorials, or case reports. The papers were also excluded if no effect estimate was reported or not enough raw data for ORs and 95%CIs calculation was available. In the case of multiple studies with the same or overlapping data published by the same researchers, we selected the most recent study with the largest number of participants. All papers meeting the criteria defined above were included for further analysis.

The literatures included were carefully reviewed for information about the first author, publication year, country, population, sample size, sex, age, detection methods of *H. pylori* and osteoporosis, diagnosis location, diagnosis, and adjusted covariates.

If data could be acquired from the tabulated literature search results, they would be extracted carefully into 2x2 tables from all eligible publications by two independent reviewers. If data were not directly available, they would be calculated from published positive predictive values and/or negative predictive values if appropriate. The adjusted OR (95%CI), if existed, was adopted instead of rude OR (95%CI)[29]. In addition, for the studies comparing the BMD of participants with and without *H. pylori* infection, the data on BMD was also extracted.

Quality assessment

Quality assessment was performed using the Newcastle-Ottawa quality assessment scale(NOS)[30]. Two researchers conducted blinded quality assessment of the included literatures. The NOS assigns a maximum of 9 points to studies of highest quality according to three quality parameters: selection, comparability, and outcome.

Statistical analyses

The primary measures were ORs and 95%CIs for the association between *H. pylori* infection and osteoporosis, and standardized mean difference (SMD) for BMD alterations between *H. pylori* positive and negative participants. To assess heterogeneity among the studies, we calculated the Cochran's Chi-squared test (with $P < 0.10$ indicating statistically significant heterogeneity) and the statistic I^2 (The heterogeneity might not be important with I^2 of 0 to 40%, while moderate heterogeneity with I^2 of 30 to 60%, substantial heterogeneity with I^2 of 50 to 90% and considerable heterogeneity with I^2 of 75 to 100%)[31]. The pooled results were calculated using fixed effects model (Inverse Variance) if no obvious heterogeneity existed, otherwise random effects model (I-V heterogeneity) was used ($P < 0.10$ was considered indicative of obvious heterogeneity). The cumulative meta-analysis was conducted for the extracted data using a pooled random effects model with the publication year to confirm whether the effect size was affected by sample size or not. In the event of obvious heterogeneity, subgroup analysis was performed according to sex, postmenopausal or not, country, Asian or not, detection methods of *H. pylori*, detection methods of osteoporosis, and detection location of dual-energy X-ray absorptiometry (DEXA). Meta regression (using ReML methods) was also performed to explore the potential heterogeneity. Publication bias was assessed by funnel plot and Egger's test[32]. A sensitivity analysis was completed by converting the pooled results from random effects model into fixed effects model or from fixed effects model into random effects model. All statistical analyses were performed using Stata 12.0.

Patient and public involvement

There was no patient and public involvement as this was a database research study.

Results

Search results

Using our search strategy, a total of 1720 articles were identified through PubMed, Web of Science, Embase and CBM, and one additional record was identified through other sources. Then, 443 duplicate papers were removed firstly, and 1242 papers were excluded after scanning their titles and abstracts. After screening the full texts of the included articles, 15 studies were excluded for the following reasons: duplicate publication(n=4), not about the topic(n=2), no related data about *H. pylori* and/or osteoporosis(n=4), lack of access to full text (n=5). A total of 21 studies [2 16 21-27 33-44] were included for further analysis (Figure 1).

Study Characteristics

A total of 21 articles were included in this study. Of the 21 articles included, 20 provided data for association between *H. pylori* and osteoporosis[2 16 21-27 33-41 43 44], 4 for the BMD alterations in *H. pylori* positive participants compared with negative controls[23 34 35 42], 3 provided both[23 34 35]. All these studies were published from 2005 to 2018. Four studies were conducted in China, 3 in Iran, 1 in Italy, 9 in Japan, 2 in Brazil, 1 in Korea and 1 in Turkey. As to the sex of participants, 4 were postmenopausal women, 4 were females, 4 were males, 9 involved both males and females. The detection methods of *H. pylori* were mainly ELISA and ^{13}C-urea breath test, while the detection methods of osteoporosis were DEXA and quantitative

ultrasound. As to the diagnosis, 5 were osteopenia, 13 were osteoporosis, and 2 provided decreased BMD (treated as osteopenia for analysis) (Table 1). In addition, 12 studies showed no significant associations of *H. pylori* infection and osteoporosis (or osteopenia), while 8 showed significant associations.

Quality evaluation

The Newcastle-Ottawa scale (NOS) was adopted to evaluate the quality of these case-control studies. Among the selection items, the evaluation results ranged from 4 to 8, with the median NOS score was 6, indicating a medium quality of the studies included. The most common source of bias came from selection and comparability. (Table 1)

Synthesis of the results

As shown in Figure 2, the overall OR was obtained based on the 20 studies involving the *H. pylori* and osteoporosis (including osteopenia) (a total of 8788 patients and healthy controls). As the existence of obvious heterogeneity (Chi-square = 69.60, I^2 = 72.7%, $P < 0.01$), random effect model was used and the pooled results of OR and its 95%CI were 1.37(1.11,1.69), indicating *H. pylori* infection was significantly associated with increased odds of osteoporosis/osteopenia. A cumulative meta-analysis was conducted with publication year in ascending order, and the results indicated that the pooled OR (95% CI) started to show statistical significance at 1.57 (95% CI: 1.02,2.41) from the ninth analyzed study, with gradually stabilizing results afterwards (Figure 3).

Subgroup analyses

Given that obvious heterogeneity existed, subgroup analyses were performed based on the potential confounding factors. All 20 studies were involved in these subgroup analyses. Figure 4 showed that both osteoporosis and osteopenia were significantly associated with *H. pylori* infection with OR(95%CI) of 1.61(1.11, 2.32) and 1.22(1.07, 1.39) respectively. Although the OR was a little higher in osteoporosis group, the meta regression analysis showed no significant difference between these two groups (t=1.18, *P*=0.26). Therefore, we pooled osteoporosis and osteopenia together to analyze other confounding factors.

Results of subgroup analyses by other factors were shown in Table 2. We found that the association between *H. pylori* infection and osteoporosis was significant in males and both sexes, but not in females. However, meta regression analysis showed no significant difference between these two groups. Moreover, no significant associations between *H. pylori* infection and osteoporosis were observed in either the postmenopausal women or non-postmenopausal women subgroup. When stratified by countries, we found significant associations between *H. pylori* infection and osteoporosis in China, Japan, and Korea (three East Asian countries). Other factors that may affect the results were presented in Table 2.

Publication bias and sensitivity analyses

Funnel plot was used to examine the publication bias of this meta-analysis. As shown in Figure 5, the funnel plot indicated no publication bias, which was also confirmed by Egger's test, with t of 1.57 and *P* of 0.13 in Figure 6. Sensitivity analysis was also performed by converting the pooled model from the random effects model to the fixed

effects model. The result of fixed effects model was 1.21(1.10-1.33), showed no obvious differences compared with the result of random effects model, indicating the pooled results was relatively stable.

Alterations of BMD in *H. pylori* infected population

Four studies were involved in this meta-analysis[23 34 35 42]. As each study included for this analysis has two different DEXA detection locations, we carried out the subgroup analysis based on detection locations, and the patients are not being counted twice in each subgroup. In addition, we chose standardized mean difference as pooled outcome for the detection methods varied with studies. As shown in Appendix S2, the BMD(g/cm^2) alterations between *H. pylori* positive and negative participants were -0.01(-0.45,0.42) for hip, -0.94(-3.15,1.28) for lumber and -0.04(-0.40,0.31) for femur using random effects model as obvious heterogeneity existed. No significant associations were observed so far.

Discussion

Although osteoporosis isn't a deadly disease, it causes huge burden to individuals and society owing to its high morbidity. Here, we got a comprehensive result by meta-analysis, indicating that *H. pylori* infection may be a risk factor for osteoporosis. However, the mechanism is still unclear. Several possible mechanisms may explain this result. First, *H. pylori* infection may lead to systemic inflammation, and release of cytokines, such as tumor necrosis factor-alpha, interleukin-1 and interleukin-6[45], which may cause bone turnover indirectly. Second, many studies have shown that low

vitamin B12 may be associated with *H. pylori* infection[46]. If the serum vitamin B12 levels are decreased, the folate becomes trapped as methyltetrahydrofolate and interrupts for folate-related DNA synthesis, which is an important factor for bone remodeling. Therefore, the decrease of vitamin B12 may lead to decreased BMD and osteoporosis[47]. Third, *H. pylori* infection may decrease the calcium absorption by causing the gastric mucosal atrophy and decreasing acid secretion. Thus, eradication of *H. pylori* may increase calcium absorption and stop the process of osteoporosis through decreasing the levels of inflammatory cytokines and improving gastric mucosal atrophy.

The present meta-analysis of 20 studies indicated that patients with *H. pylori* infection were associated with an estimated 1.37 times higher odds ratios of developing osteoporosis as compared with those without *H. pylori* infection, while no associations were found in previous meta-analysis [19 20] (one had 5 studies involving 1321 participants, and one had 4 studies involving 520 participants). As the previous meta-analysis studies had no quality assessment and our analysis included more studies and participants, the results in our study might be more reliable than the previous meta-analysis studies.

Despite the significant association between *H. pylori* infection and osteoporosis, obvious heterogeneity existed between the included studies. We found that sex of participates may affect the results. As known to all, female and postmenopausal women are independent risk factors of osteoporosis. Here, we explored the relationship between osteoporosis and *H. pylori* infection, and found that the

relationship was significant in male, but not in female (whether postmenopausal or not), which wasn't paradoxical with the fact that women with postmenopausal should have a higher risk of osteoporosis than men. In the group of both sexes, the results showed statistic difference and obvious heterogeneity, which may be due to the ratio of M/F and other confounding factors. Therefore, we might suggest that more attention should be paid to male than female in *H. pylori* positive patients. However, only 7 studies (4 were about postmenopausal women and 3 were about non-postmenopausal women) were conducted in female, the results may be not that reliable due to the small sample size. Another reason may also be possible, that the different degree of osteoporosis may affect the diagnosis and some early patients may be regarded as healthy controls. Further studies with dose-response relationship of different severity of osteoporosis and prevalence may help to confirm this hypothesis. In the subgroup analysis by criteria (osteoporosis and osteopenia), the OR in osteoporosis was a little higher than that in osteopenia, which may also help to prove our hypothesis. In the subgroup analysis based on countries, significant association was evidenced in three East-Asian countries (China, Japan and Korea are from East Asia), indicating many other factors associated with geography may affect the results. As only two studies were non-Asian countries, the reason of this phenomenon may be due to the sample size, the same situations also happened in other Asian countries with only one study included. In addition, as most studies included in our studies were Asian countries, especially East Asia, whether our findings can be applied to other populations around the world needs further exploring.

In our research, we also explored the heterogeneity from diagnosis methods factors. We found that the detection methods of osteoporosis (DEXA and quantitative ultrasound) affected the pooled results, and the detection locations of DEXA also contributed to the heterogeneity. From our results, we thought that DEXA might be a better tool to diagnose osteoporosis in assessing the association between *H. pylori* and osteoporosis. The same situation also happened in the detection methods of *H. pylori*. We found ELISA and multi-method strategy may provide more homogeneous results. In total, in despite of the significant association between *H. pylori* and osteoporosis, as the heterogeneity still existed obviously, further studies were still needed to address its potential confounding factors.

In a previous meta-analysis study, Karn Wijarnpreecha et al found increased odds of nonalcoholic fatty liver disease (NAFLD) among patients infected with *H. pylori* [48]. However, Sikarin Upala et al found that no significant difference in BMD between patients with fatty liver disease and controls[49]. Combine the two meta-analysis and our results, we may guess that *H. pylori* may be an independent risk factor of NAFLD and osteoporosis, and/or *H. pylori* infection may be an important confounding factor in exploring the relationship between NAFLD and osteoporosis, or no actual relationship between NAFLD and osteoporosis exists. However, as the authors stated[49], the review was a preliminary result because of limited amount of literature, it might be too early to have definite conclusion.

We also compared the quantitative alterations of BMD in *H. pylori* infected subjects. However, no significant difference was found. The reason may be that: 1) the sample

size was relatively small, 2) the severities of *H. pylori* infection were not serious, or the infection of *H. pylori* didn't last long enough to cause alterations, 3) though the basic characteristics of included studies were comparable, many other confounding factors that might affect BMD have not been adjusted. Therefore, more studies with large sample size were still needed to verify the alterations of BMD in *H. pylori* infection.

The strength of the present meta-analysis lies in inclusion of 21 observational studies reporting data on *H. pylori* infection and osteoporosis, and the alterations of BMD by *H. pylori*. However, our meta-analysis has several limitations that should be recognized when interpreting the results. First, most of the included studies were hospital-based or health center-based, which were not affected by detection bias, but might be subjected to selection bias. However, the prevalence of *H. pylori* infection in most studies that we selected was consistent with the incidence rate in the general population. Second, our analysis had an ascertainment bias that might be present because progression of osteoporosis is continuous, and some patients may be classified as controls. However, this may lead to a more conservative result, which may help to indicate that our overall result is reliable. Third, the heterogeneity is still obvious. However, we performed subgroup analyses based on study characteristics, and found that some factors may affect the association. In addition, when available, adjusted estimates were used in preference to unadjusted estimates. Even though the adjusted estimates may be closer to the true effect for adjusted results could control confounding factors[50 51], the different adjusted factors in different studies may also

contribute to the heterogeneity. Four, the qualities of included studies were medium, and some studies were published informally. We also included all these studies based on inclusion and exclusion criteria to avoid publication bias. Nevertheless, our study is still the most comprehensive about the association between *H. pylori* infection and osteoporosis.

In summary, our results suggest significant increased odds of osteoporosis in patients with *H. pylori* infection. The clinicians should pay more attentions to the patients infected with *H. pylori* by using DEXA scan, especially those chronic gastritis patients. However, the results should be cautiously interpreted considering the heterogeneity and the fact that all studies are non-randomized and retrospective. Further studies are needed to explore the mechanism and confounding factors between *H. pylori* and osteoporosis.

Funding

This work was supported by the National Natural Science Foundation of China [No. 81202220 to Y. Z.; No. 81673211 and 81372952 to T. C.], by the Natural Science Foundation of Jiangsu Province, China [No. BK20150094 to Q.Z.], and by the Chongqing Health and Family Planning Commission [No. 20141027 to H. X.].

Contributors

YZ and HX led the study by designing, interpreting results, and revising manuscript critically for important intellectual content; TW and XL contributed to data analysis, result interpretation and drafting of the manuscript; QZ, BG and JZ participated in

study data collection and revising manuscript; TW, XL, TC and LY participated in study conduct and results interpretation. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Patient consent Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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Figure legend

Figure 1. Flow diagram of the article selection for systematic review.

Figure 2. Forest plot of the included studies assessing the association between *Helicobacter pylori* and osteoporosis (random effects models). CI indicates confidence interval.

Figure 3. Forest plot of cumulative meta-analysis.

Figure 4. Forest plot of subgroup meta-analysis according to diagnosis (random effects models).

Figure 5. Funnel plot of publication bias for the association between *Helicobacter pylori* and osteoporosis.

Figure 6. The Egger's test for publication bias.

Table 1. Characteristics and quality assessment of the studies included

Author [Reference]	Year	Country	Sex(M/F)	Age(mean age±SD or (range age) years)	Detection method of *H.pylori*	Detection methods of osteoporosis	Diagnosis locations	Diagnosis	Cases/Controls/ Total	Scores of NOS	Main adjusted factors (the methods used for adjusting)
Figura, N.[44]	2005	Italy	Males	65 (55–82) for patients; 64.5(55–80) for controls	ELISA	DEXA	Lumbar and femur bone	Osteoporosis	80/160/240	7	age, socioeconomic background, and smoking habits.
Adriana M. KAKEHASI[43]	2007	Brazil	Postmenopausal women	61.6±7(50–79)	non-ELISA	DEXA	Lumbar spine	Osteoporosis	18/32/50	6	mean age, body mass index, age at menarche, postmenopausal period
Adriana M. Kakehasi[42,#]	2009	Brazil	Postmenopausal women	63.7±7.3 for Hp(+) 62.5±7.0 for Hp(-)	non-ELISA	DEXA	Lumbar spine and hip	Not fitted*	34/27/61	-	age, Postmenopausal time, BMI
Akkaya, Nuray[41]	2011	Turkey	Postmenopausal women	65.29±6.09 patients; 63.57±6.53 controls	ELISA	DEXA	Lumbar and femur neck	Osteoporosis	58/47/105	6	age, education level, occupation, age of menarche or menopause, duration of postmenopausal, period or daily consumption of tea, coffee, alcohol or dairy products
Chinda, D.[40]	2013	Japan	379/631	not mentioned	ELISA	QU	Calcaneal osteo	Osteopenia	-/-/1010	7	age, BMI, smoking, alcohol consumption, periodical exercise, latest educational level (logistic regression analysis)
Asaoka, Daisuke[39]	2014	Japan	95/105	63.1±8.8 years	Both	DEXA	Lumbar vertebrae	Osteoporosis	41/159/200	6	age, gender, BMI, alcohol consumption, smoking, BAP, PUD, and EGA (multivariate logistic regression analysis)
Asaoka, D.[38]	2014	Japan	131/26	71.1±7.5 patients 61.6±8.9 controls	not mentioned	DEXA	Lumbar	Osteoporosis	24/133/157	6	age, sex, BMI, Brinkman Index (B.I.), accmulated amount of alcohol (multivariate analysis)
Lin, S. C.[37]	2014	China	Female	77 (65–97)	non-ELISA	DEXA	Not mentioned	Osteoporosis	101/264/365	5	age group, body mass index group, and use of proton pump inhibitor (multivariate logistic regression analyses)
Asaoka, D.[36]	2015	Japan	130/134	69.8±6.8 for patients 61.9±8.2 for controls	not mentioned	DEXA	Not mentioned	Osteoporosis	45/219/264	7	age, sex, BMI. etc (multivariate analysis)
Asaoka, D.[16]	2015	Japan	120/135	63.2±8.5	Both	DEXA	Lumbar vertebrae	Osteoporosis	43/212/255	6	age, sex, BMI, cumulative alcohol intake, Brinkman index, type 2 diabetes mellitus, calcium channel blocker, PPI, hemoglobin, calcium, gamma glutamyl transpeptidase, bone-specifi alkaline phosphatase, NTX, hiatal hernia, and EGA (multivariate logistic regression analysis)
Chung, Y. H.[35,#]	2015	Korea	Men	54.4±10.7 for Hp+ 51.9±12.1 for Hp-	ELISA	DEXA	Lumbar (L1–L4)	osteopenia	-/-/1126	7	Height, weight, BMI, alcohol, exercise.
Fotouk-Kiai, M.[34,#]	2015	Iran	575/392	68.3±6.8 for hp+ 69.3±7.4 for hp-	ELISA	DEXA	Lumbar vertebra	Osteoporosis	314/653/967	5	age, sex, smoking, alcohol consumption and BMI

							and Femur				
Mizuno, S.[33]	2015	Japanese	Men	62.1±5.0 for low TBD 58.4±5.7 for normal	ELISA	QU	Not mentioned	Decreased BMD	116/114/230	8	Age, BMI and smoking habit (logistic regression analysis)
Chinda, D.[21]	2016	Japan	Men	50.2±15.4 years	ELISA	QU	Not mentioned	Osteopenia	-/-/295	7	age, BMI, serum level of estradiol, the intake of calcium per day, smoking, drinking, periodical exercise, last educational background (logistic regression)
Chinda, D.[22]	2016	Japan	Females	52.2±15.2	ELISA	QU	Not mentioned	Osteopenia	-/-/473	6	age, BMI, smoking, alcohol consumption, periodical exercise, last educational level, serum level of estradiol, calcium intake per day (multiple logistic regression)
Kalantarhormozi, M. R.[23,#]	2016	Iran	Postmenopausal women	58.87±8.02	ELISA	DEXA	lumbar spine and femur	Osteoporosis	16/234/250	6	age and BMI (multiple linear regression)
Zhang, Chaoxian[24]	2016	China	194/126	38.32±10.64 for patients 38.27±7.46 for controls	non-ELISA	DEXA	Not mentioned	Osteoporosis	160/160/320	5	Age, gender, gene (multiple logistic regression)
Abdolahi, N.[25]	2017	Iran	Postmenopausal women	not mentioned	ELISA	not mentioned	Not mentioned	Osteoporosis	73/34/107	8	not mentioned
Chinda, D.[26]	2017	Japan	Females	62.5±8.6 for patients 44.9±10.9 for controls	ELISA	QU	Calcaneus	Osteopenia	197/276/473	4	age, smoking and drinking habit, schooling duration, estradiol levels, menopause, birth history (multiple logistic regression analysis)
Lu, Li-juan[2]	2018	China	1474/393	54.0±9.6	non-ELISA	QU	Calcaneus	Osteoporosis	900/967/1867	6	gender and age
Pan, B. L.[27]	2018	China	568/299	55.9±11.3	non-ELISA	DEXA	Not mentioned	Decreased BMD	311/556/867	5	Sex, age, BMI, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and peptic ulcer disease (multiple stepwise logistic regression analyses)

Not fitted *: this study only explored the alteration of BMD in patients with *H. pylori* infection.

#: this study also reported the BMD in patients with *H. pylori* infection.

ELISA: enzyme linked immunosorbent assay; QU: quantitative ultrasound; DEXA: dual energy X-ray absorptiometry scan; NOS：Newcastle-Ottawa scale

Table 2. Overall effect estimates for *Helicobacter pylori* infection and osteoporosis according to study characteristics

Factors	Categories	No. of studies	OR [95%CI]	Model used	Heterogeneity		Meta-regression	
					I^2	*P*-value	t	*P*-value
Sex*								
	Female	8	1.09(0.87,1.35)	Fixed	33.0%	0.17	-	-
	Male	5	1.27(1.07,1.50)	Fixed	14.6%	0.32	0.47	0.64
	Both	9	1.21(1.07,1.37)	Random	85.6%	0.00	1.78	0.09
Postmenopausal or not								
	Non-postmenopausal women	4	1.08 (0.83,1.41)	Fixed	48.0%	0.12	-	-
	Postmenopausal women	4	1.09(0.75,1.58)	Fixed	35.8%	0.20	-0.13	0.90
Country								
	China	4	1.86(1.06,3.28)	Random	90.4%	0.00	-	-
	Japan	9	1.57(1.08,2.28)	Random	63.7%	0.005	-0.39	0.70
	Italy	1	0.87(0.50,1.53)		-	-	-1.11	0.29
	Brazil	1	0.42(0.12,1.42)		-	-	-1.69	0.11
	Korea	1	1.29(1.05,1.57)		-	-	-0.59	0.57
	Iran	3	1.06(0.60,1.86)	Random	61.3%	0.075	-1.16	0.27
	Turkey	1	0.95(0.53,1.69)		-	-	-0.98	0.34
Asian country or not								
	Non-Asian country	2	0.77(0.46,1.28)	Fixed	12.8%	0.28	-	-
	Asian country	18	1.44(1.16,1.79)	Random	73.9%	0.00	1.60	0.13
Detection methods of *H. pylori*								
	ELISA	11	1.09(0.96,1.24)	Fixed	32.1%	0.14	-	-
	Non-ELISA	5	1.62(0.96,2.72)	Random	88.4%	0.00	1.52	0.15
	Both	2	3.67(1.88, 7.16)	Fixed	0%	0.42	2.65	0.02
Detection methods of osteoporosis								
	DEXA	13	1.58(1.14,2.18)	Random	79.5%	0.00	-	-
	QU	6	1.05(0.90,1.22)	Fixed	0%	0.51	-1.33	0.20
Detection location of DEXA								

	Lumbar	6	1.75(0.99,3.07)	Random	65.4%	0.013	-	-
	Femur	3	1.56(1.17,2.08)	Fixed	0%	0.90	-0.17	0.87

*: One study has both males, females and over results, it was used three times in subgroups analysis (males, females, and both).

ELISA: enzyme linked immunosorbent assay; QU: quantitative ultrasound; DEXA: dual energy X-ray absorptiometry scan.

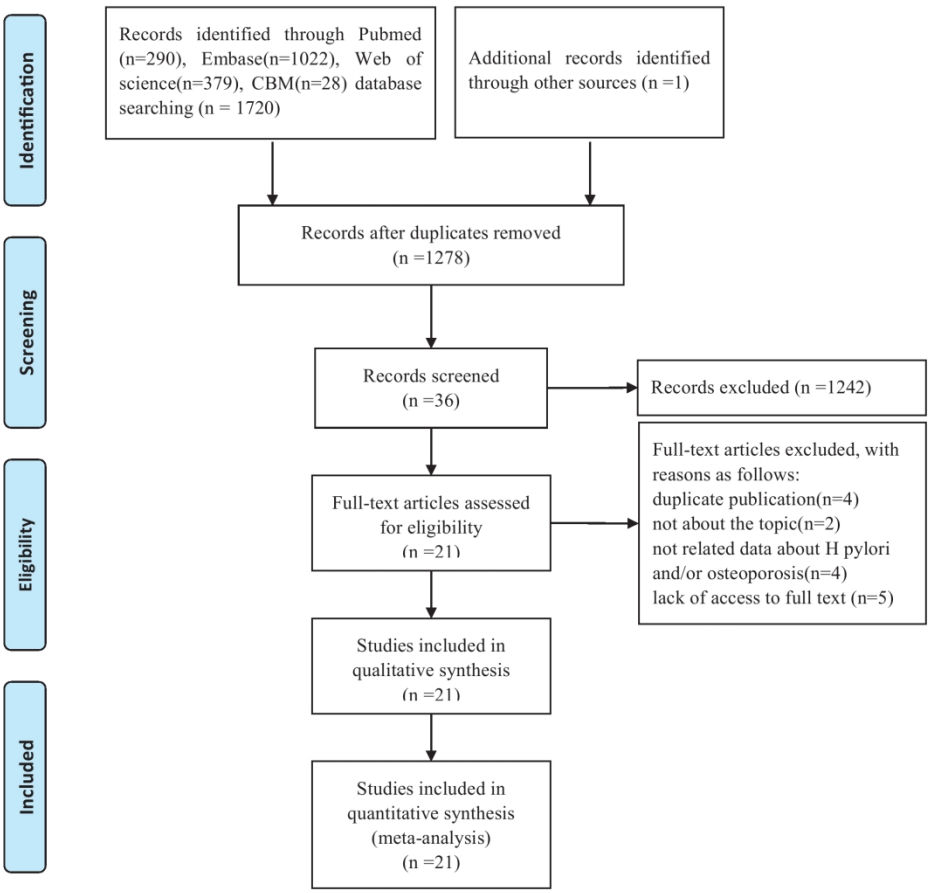


Fig 1

199x194mm (300 x 300 DPI)

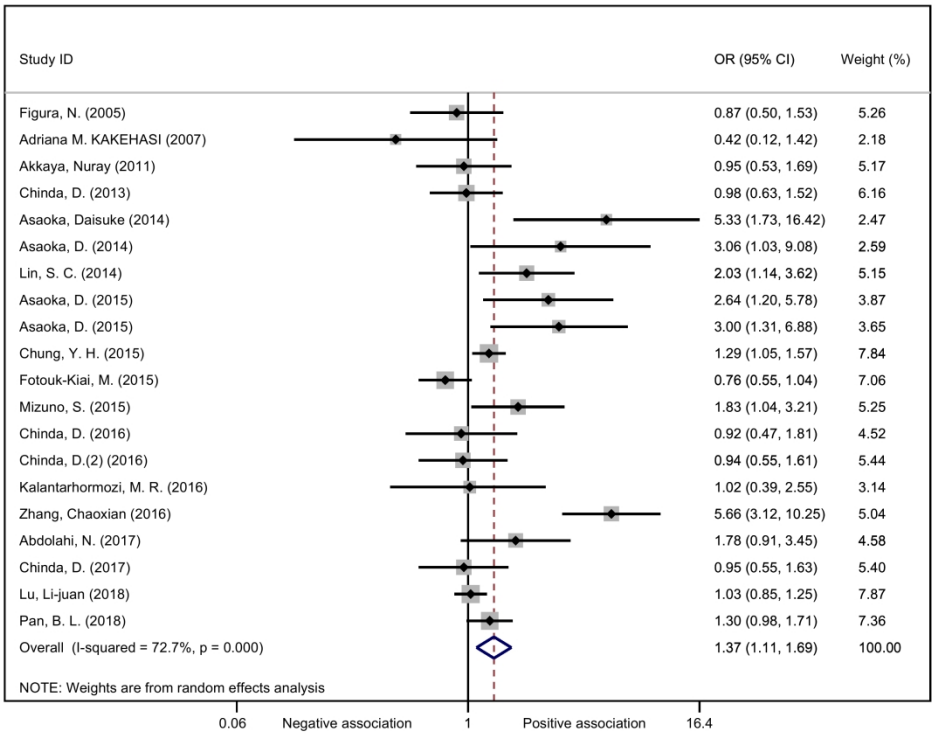


Fig 2

279x228mm (300 x 300 DPI)

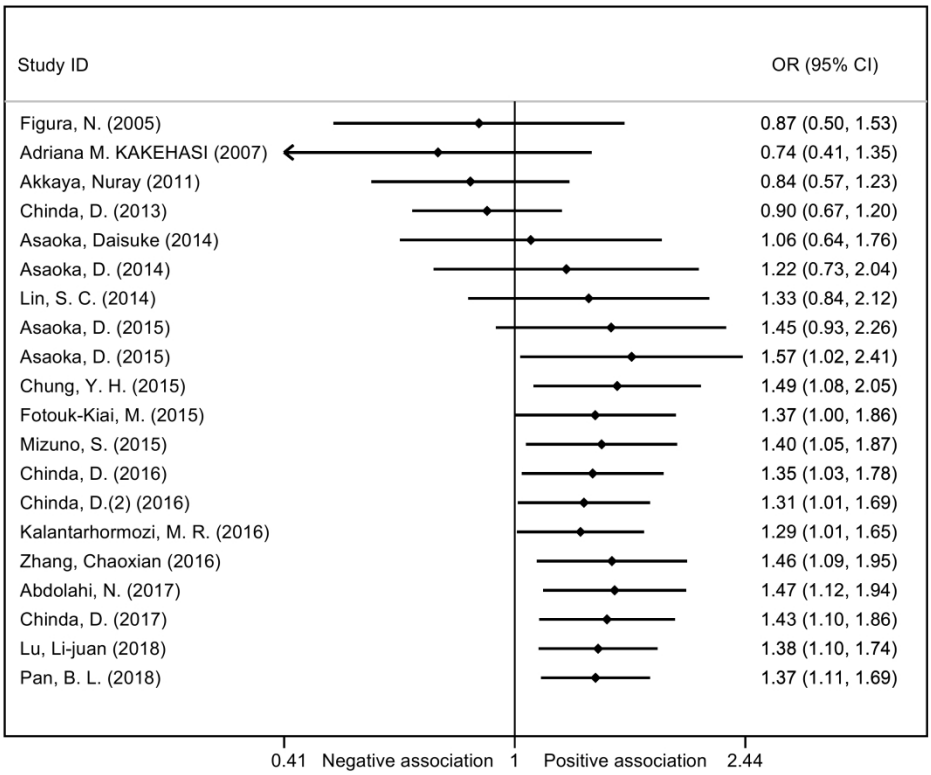


Fig 3

281x240mm (300 x 300 DPI)

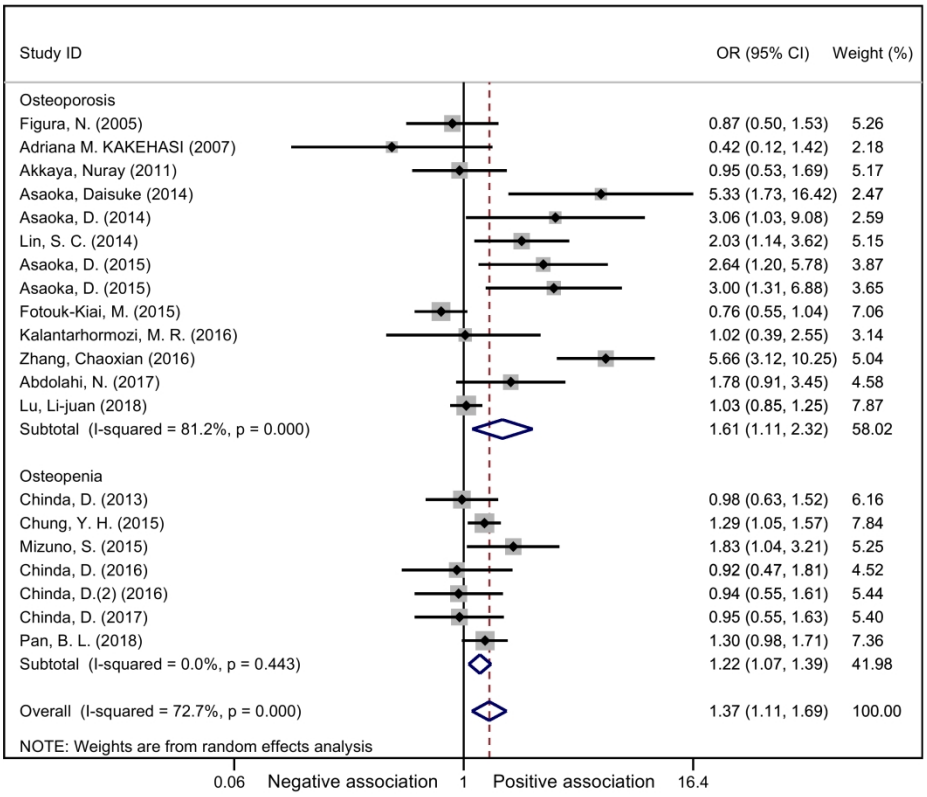


Fig 4

282x248mm (300 x 300 DPI)

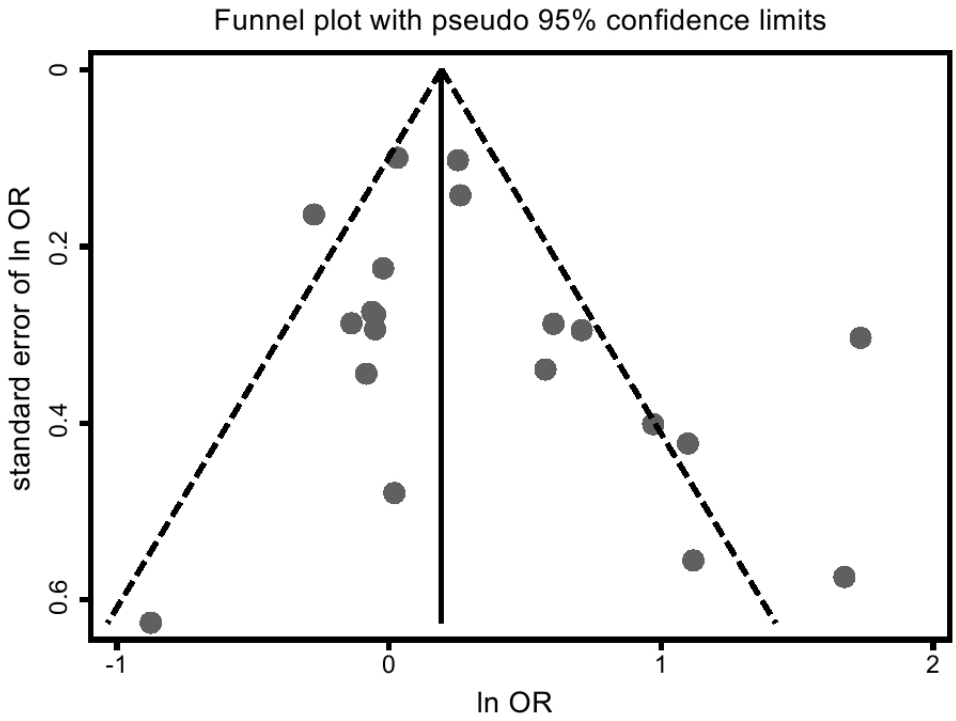


Fig 5

79x60mm (300 x 300 DPI)

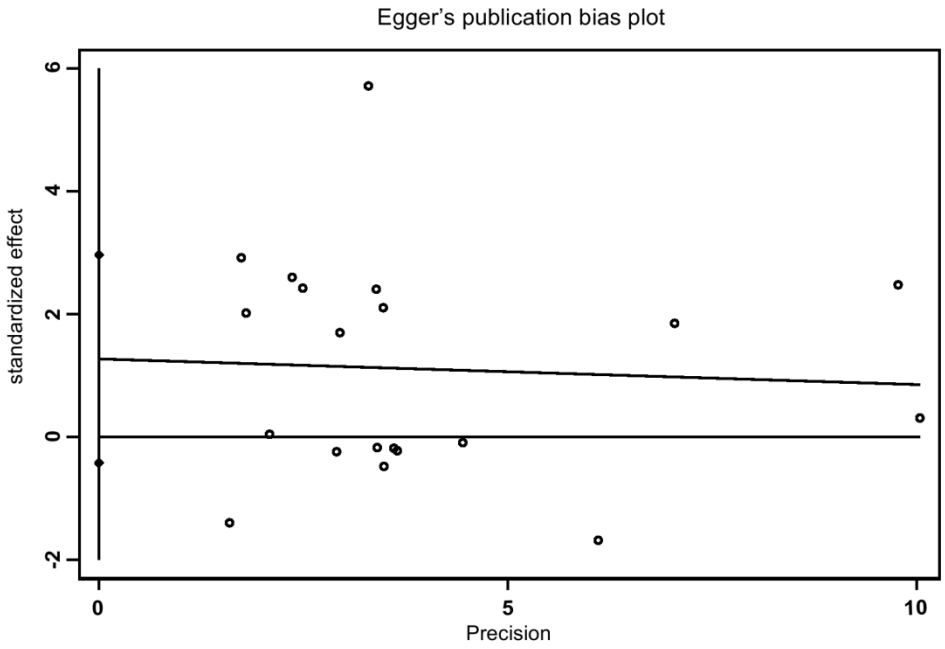
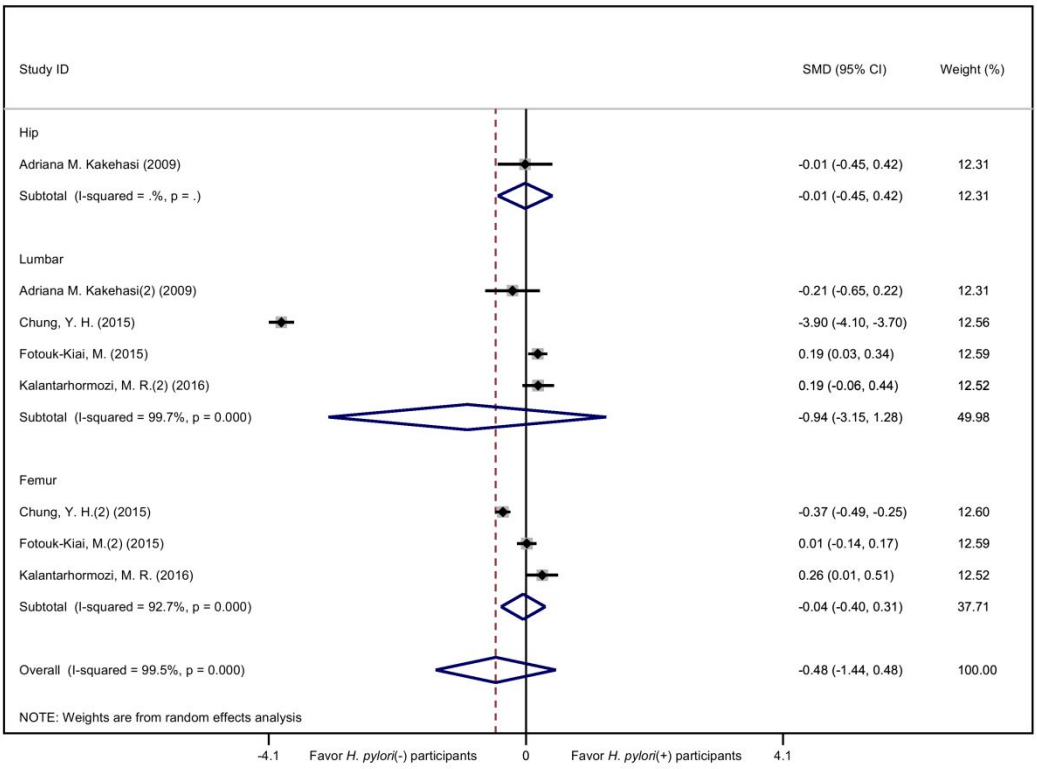


Fig 6

152x101mm (300 x 300 DPI)

The search string for our study:

The search strategy for H. pylori were "((helicobacter pylori) OR (campylobacter pylori) OR (H. pylori) OR (h. pylori) OR hp OR helicobacter OR (helicobacter bill) OR (helicobacter hepaticus) OR (helicobacter pullorum) OR (h. bilis) OR (h. hepaticus) OR (h. pullorum) OR (h. ganmam) OR (helicobacter species) OR (helicobacter sp) OR (helicobacter genus) OR campylobacter OR (campylobacter infection) OR campylobacteriosis OR (helicobacter pylori infection) OR (helicobacter infection) OR (pylori) OR (enterohepatic helicobacter spp) OR (campylobacter spp)), the search strategy for osteoporosis were ("fragility fracture" OR "Bone Density" [MeSH] OR "bone density" OR "bone mass density" OR "Osteocalcin" [MeSH] OR "bone loss" OR "Osteoporosis" OR "Osteoporosis" [MeSH])". Finally, we used "AND" to pool these results.



Meta-analysis of SMD according to detection locations.