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BRIEF ARTICLE

Does *Helicobacter pylori* infection play a role in iron deficiency anemia? A meta-analysis

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Abstract

AIM: To perform a meta-analysis of observational studies and randomized controlled trials (RCTs) on the association between *Helicobacter pylori* (*H. pylori*) and iron deficiency anemia (IDA).

METHODS: A defined search strategy was used to search Medline, Embase, the Cochrane Library, Clinical Trials, Cochrane Central Register of Controlled Trials, Premedline and Healthstar. Odds ratio (OR) was used to evaluate observational epidemiology studies, and weighted mean difference (WMD) was used to demonstrate the difference between control and intervention groups.

RESULTS: Fifteen observational studies and 5 RCTs were identified and used for calculation. The pooled OR for observational studies was 2.22 (95% CI: 1.52-3.24, $P < 0.0001$). The WMD for hemoglobin (HB) was 4.06 g/L (95% CI: -2.57-10.69, $P = 0.01$), and the WMD for serum ferritin (SF) was 9.47 μg/L (95% CI: -0.50-19.43, $P < 0.0001$). Results were heterogeneous for all comparisons.

CONCLUSION: This meta-analysis on observational studies suggests an association between *H. pylori* and IDA. In RCTs, eradication of *H. pylori* can improve HB and SF levels but not significantly.

Key words: *Helicobacter pylori*; Iron-deficiency anemia; Meta-analysis; Hemoglobins; Odds ratio

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INTRODUCTION

Anemia, defined as a hemoglobin concentration below established cut-off levels, is a widespread public health problem with major consequences for human health as well as social and economic development[1]. The World Health Organization (WHO) estimates that about 2 billion people in the world are suffering from this disease, and that approximately 50% of all anemia cases are diagnosed as iron deficiency anemia (IDA)[2,3]. IDA affects the work capacity of patients and may contribute to mortality, thus limiting economic development. The overall death rate from IDA has been underestimated in most surveys from many developing and developed countries[4,5]. WHO suggested that researchers and clinical doctors should investigate the etiology of IDA and develop therapeutic strategies because timely treatment restores personal health and increases national productivity by 20%[6,7].

It is known that a variety of causes such as inadequate iron intake, chronic blood loss, chronic disease, malabsorption, hemolysis, or a combination of these, can induce IDA[5,8-10]. Among possible causes, the involvement of *Helicobacter pylori* (*H. pylori*) infection remains controversial[11-13]. *H. pylori* is a highly prevalent microbial infection. Over 50% people in the world are infected by *H. pylori*. In Africa, Mexico, South America and Central America, *H. pylori* infection reaches 70%-90% of the population[14,15]. *H. pylori* has been considered as a major cause for the development of peptic ulcer disease, gastric malignancy and dyspeptic symptoms[16-19]. Recent studies have shown that *H. pylori* can also cause other extragastric diseases[20-22]. However, knowledge regarding any relation between *H. pylori* infection and IDA is limited. Moreover, studies regarding the role of *H. pylori* infection in IDA and the effectiveness of the eradication of *H. pylori* in the treatment of IDA are controversial.

This clinical research question is addressed by this meta-analysis. The aim of the study was to evaluate the association between *H. pylori* infection and IDA and examine the effect of *H. pylori* eradication on serum hemoglobin (HB) and serum ferritin (SF) levels. Observational epidemiological studies have demonstrated an association between *H. pylori* and IDA by comparing IDA risk between *H. pylori*-infected and non-infected participants. Randomized controlled trials (RCTs) have established a cause and effect relationship between *H. pylori* and IDA. In this meta-analysis, we hypothesized that there is a significant difference in IDA risk between *H. pylori*-infected and non-infected participants, and that *H. pylori* eradication therapy can significantly increase HB and SF concentration, thus alleviating IDA. We tested our hypothesis by pooling the results of studies on *H. pylori* and IDA.

MATERIALS AND METHODS

Search strategy and identification of studies

We searched, without language restrictions, for all publications on *H. pylori* and IDA between January 1966, and June 2009. Searches were performed on Medline, Embase, Clinical Trials, Database of Abstracts of Reviews of Effects (DARE), Cochrane Central Register of Controlled Trials (CENTRAL), the Cochrane Database of Systematic Reviews, Premedline, Healthstar, by using the MeSH heading: "*Helicobacter pylori*", "iron-deficiency anemia", "anemia", "iron" and "hemoglobin" and the non-MeSH terms "sideropenic refractory anemia" and "serum ferritin". The reference lists of major textbooks, review articles, and of all the included articles identified by the search were then individually searched to find other potentially eligible studies. Information about unpublished and ongoing RCTs was sought from authors of the included RCTs, and experts in the field.

Selection criteria and validity assessment

The present meta-analysis followed the Quality of Reports of Meta-Analyses of RCTs (QUOROM) guideline for RCTs and observational studies in epidemiology [Meta-analysis of Observational Studies in Epidemiology (MOOSE) and Methodological Index for Non-Randomized Studies (MINORS)] guideline in observational studies[23-25]. To avoid selection bias, selection criteria were established before searching. Two reviewers (Qu XH and Huang XL) identified articles eligible for further review by performing an initial screen of the abstracts or titles of the search results. The second screening was based on a full-text review according to the selection criteria. The observed agreement between reviewers for eligibility of articles was 96.3%, corresponding to modest agreement (κ = 0.40). Discrepancies were resolved by discussion and consultation with other reviewers (Xiong P and Zhu CY).

Observational epidemiology studies: Observational epidemiology studies (cross-sectional, case-control, or cohort) investigating the prevalence of IDA in *H. pylori*-positive patients and negative controls were included in this meta-analysis. Duplicate publications and those studies in which patients had other underlying common IDA causes (e.g. aspirin/NSAID use, colonic carcinoma, gastric carcinoma, angiodysplasia) were excluded.

Randomized controlled trials: In order for an RCT to be included, its participants must have had both *H. pylori* infection and IDA/iron deficiency (ID). At least 2 authors independently assessed the methodological quality of included RCTs by Jadad scores[26]. In addition, for a study

to be eligible for inclusion, the use of therapy to eradicate *H. pylori* in intervention groups and administration of oral ferrous sulfate to both intervention and control groups were required. Discrepancies in data extraction were resolved by discussion among authors (Qu XH and Huang XL).

Data abstraction

For observational epidemiology studies, we collected information on the year of publication, location of the study, age groups, number of cases and controls, country and region, number of IDA positive and negative patients, test method for *H. pylori*.

For RCTs, we collected information on references, year of publication, sample size, age group, treatment therapies, *H. pylori* testing methods and changes in mean ± SD of HB and SF in both the intervention and control groups.

Statistical analysis

For observational epidemiology studies, we recorded the prevalence of IDA in *H. pylori*-positive patients and controls for each study as an odds ratio (OR) and 95% CI and the weight of the studies. We used the heterogeneity χ^2 (Cochran Q) statistic to formally analyze heterogeneity across included studies. Meta-analysis was performed using Review Manager Version 5 (Cochrane Collaboration and Update Software) for observational studies[27].

For RCTs, we collected changes in HB and SF concentration after *H. pylori* eradication and evaluated them by using weighted mean difference (WMD) with 95% CI. A χ^2 test was used to assess heterogeneity of the studies. If the studies were found to be heterogeneous, (i.e. χ^2 > df), we utilized the DerSimonian and Laird random-effects model[28] rather than a fixed effects model to reassess the pooled estimates. The source of heterogeneity was investigated as described below. Publican bias was performed by both Review Manager Version 5 and STATA version 10. We also performed the Duval and Tweedie nonparametric "trim and fill" procedure[29] to further assess the possible effect of publication bias in our meta-analysis.

Subgroup analysis

Subgroup analysis was performed to assess the factors that might impact the pooled estimates and to investigate the source of heterogeneity. Sensitivity analysis was also conducted to test whether the analysis was robust by changing statistical methods, reanalyzing the data, and comparing the 2 results by the *t* test.

Publication bias

Funnel plots and Begg's test[30] are thought to detect the existence of publication bias of pooled ORs within observational studies. Small studies are scattered widely at the bottom of the graph, while the spread narrows for larger studies. When a funnel plot seemed to be asymmetrical, we used Duval and Tweedie's nonparametric "trim and filled" method as a sensitivity analysis to reassess the pooled estimates[29]. This method considers the possibility of hypothetical "missing" studies that might exist and recalculates the results with the imputed missing studies.

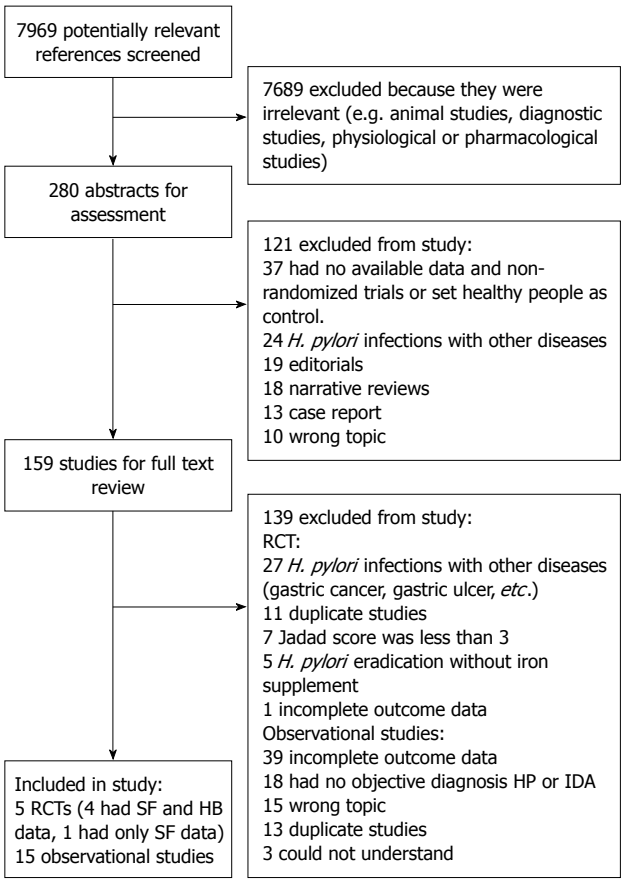

Figure 1 Flow chart showing the trial flow for selection of RCTs and observational studies to be included. RCT: Randomized controlled trial; HP: *H. pylori*; IDA: Iron deficiency anemia; HB: Hemoglobin; SF: Serum ferritin.

RESULTS

Search results

The search strategy retrieved 7969 potentially relevant references. Of these, 7689 were not relevant, e.g. animal studies, physiological or pharmacological studies. The remaining 280 references were assessed by screening their abstracts, and we excluded any references that were editorials or narrative reviews. One hundred and fifty nine studies were subjected to a full text review and excluded according to the selection criteria as described earlier. Supplementary studies were identified that had been published only as abstracts from conference proceedings of scientific meetings. We then excluded all RCTs with a Jadad score under 3 to ensure the quality of eligible trials. Fifteen observational studies[31-45] and 5 RCTs[46-49] (4 of which[46-49] provided both HB and SF data, and one study[40] provided only SF data) meeting our criteria were included in our meta-analysis (Figure 1).

Table 1 Summary characteristics of studies and participants

Ref.	Participants	Age group (yr)	Male (%)	Prevalence of *H. pylori* infection (%)		*H. pylori* test methods
Observational studies					Sampling mode	
Milman *et al*[31]	2264	30-60	1153 (51)	32.0	Hospital/health examination	Serum IgG
Choe *et al*[32]	375	10-15	205 (55)	16.8	High school/questionnaires	Serum IgG
Cuoco *et al*[33]	362	16-58	115 (32)	21.0	Hospital diagnosed patients	Histological examination
Choe *et al*[34]	660	15-17	376 (57)	29.5	High school/physical examination	Serum IgG
Nahon *et al*[35]	210	57.4 (21.4) (SD)	80 (38)	NA	Hospital diagnosed patients	Histological examination
Choe *et al*[36]	937	10-18	475 (51)	20.8	-/physical examination	Serum IgG
Choi[37]	674	9-12	344 (51)	13.6	Middle-class families/physical examination	Serum IgG
Ciacci *et al*[38]	55	> 17	22 (40)	60.0	Hospital diagnosed patients	Histological examination/rapid urease test
Hershko *et al*[39]	210	16-77	NA	NA	Hospital diagnosed patients	UBT/Serum IgG
Gessner *et al*[40]	690	7-11	NA	87.0	8 most populous villages/physical examination	UBT
Baggett *et al*[41]	668	7-11	354 (53)	86.5	10 predominantly villages/physical examination	UBT
Cardenas *et al*[42]	7462	⩾ 3	NA	27.1	NHANES/questionnaire, laboratory, examination data	Serum IgG
Süoglu *et al*[43]	70	4-16	NA	50.0	Hospital diagnosed patients	Histological examination
Haghi-Ashtiani *et al*[44]	209	2-14	111 (45)	47.8	Hospital diagnosed patients	Histological examination
Mulayim *et al*[45]	117	NA	0 (0)	61.5	Hospital diagnosed patients	UBT
Randomized controlled trials					Treatment therapies	
Choe *et al*[46]	13	10-17	NA	19.7	B+A+M	Histology
Gessner *et al*[40]	201	7-11	NA	87.0	L+A+C	UBT
Chen *et al*[47]	86	18-76	50 (58)	NA	B+A+M	UBT
Vijayan *et al*[48]	22	> 13	NA	NA	L+T+C	Rapid urease test/histology
Sarker *et al*[49]	99	2-5	46 (46)	NA	O+A+C	UBT

UBT: Urea breath test; IgG: Immunoglobulin G; NHANES: National Health and Nutrition Examination Survey; B: Bismuth; A: Amoxicillin; M: Metronidazole; L: Lansoprazole; C: Clarithromycin; T: Tinidazole; O: Omeprazole.

Study characteristics and quality

In total, 15 183 patients from 20 studies (15 observational and 5 RCTs) were included in the meta-analysis, and the characteristics of the sample are summarized in Table 1.

Of the observational studies, 4 studies with participants over 18 years old[31,35,38,39] and one study with patients aged 16-58 years old[33] were classified as adult groups. Three studies with patients aged 10-18 years old were classified as adolescent groups[32,34,36]. Two studies with patients younger than 11 years old were classified as child groups[41,49]. For the presence of *H. pylori*, 6 studies utilized serum immunoglobulin G (IgG)[31,32,34,36,37,42], 5 utilized histological examination[33,35,38,43,44] and 3 used the urea breath test (UBT)[40,41,45]. UBT and serum IgG were utilized by Hershko *et al*[39] in their studies. Of the RCTs, 2 studies with participants aged 2-11 years old were classified as child groups[40,49] and 3 were classified as adolescent and adult groups[46-48]. All the RCTs used eradication triple therapy for *H. pylori* as intervention.

Study methodological quality is shown in Table 2 (observational studies) and Table 3 (RCTs). In observational studies, the MINORS quality score ranged from 6 to 14 points. Only 8 (53%) of the articles included employment outcomes as part of the main study aim. Five studies satisfied the criteria for inclusion. Most data were collected according to a protocol established before the beginning of the study, and most studies have no exclusion or details about the reasons for exclusion. In RCTs, qualities of all the studies were evaluated by Jadad sore. All of the studies included had a score greater than 3. Only one study fulfilled all of the evaluated quality criteria. All studies were randomized, and for 4 of them, the generation of allocation sequence was judged adequate. Only 2 studies were designed as double-blind, but placebo was offered in only one study. Three of the 5 RCTs had no exclusions, and one of the 5 RCTs gave details on the reasons for exclusion.

Summary estimates

Risk of IDA for *H. pylori*-positive *versus H. pylori*-negative patients: We tested the heterogeneity of the 15 observational studies that provided information about prevalence OR, and the heterogeneity χ^2 statistic was 51.29 ($P < 0.00001$). Therefore, the pooled estimates were evaluated under a random effects model instead of a fixed effects model. The pooled OR was 2.22 and the 95% CI was 1.52-3.24 ($P < 0.0001$) suggesting that IDA is associated with *H. pylori* (Figure 2).

***H. pylori* eradication effect in IDA patients:** Four RCTs[46,48-49] reported blood parameter (HB levels and SF concentrations) differences and one RCT[40] reported

Table 2 Methodological quality assessment based on MINORS[1]

Source	Aim[2]	Rate[3]	Data[4]	Measure[5]	Bias[6]	Time[7]	Loss[8]	Size[9]	Total[10]
Milman *et al*[31]	2	2	2	2	2	1	1	0	12
Choe *et al*[32]	2	1	1	1	2	1	0	0	8
Cuoco *et al*[33]	1	1	1	2	2	0	0	1	8
Choe *et al*[34]	2	1	1	2	2	1	0	0	9
Nahon *et al*[35]	1	2	2	1	2	1	1	0	10
Choe *et al*[36]	2	2	1	1	0	0	0	0	6
Choi[37]	2	1	1	2	2	2	1	0	11
Ciacci *et al*[38]	1	1	2	1	2	1	0	0	8
Hershko *et al*[39]	1	1	1	1	2	1	1	0	8
Gessner *et al*[40]	2	2	2	1	2	2	2	1	14
Baggett *et al*[41]	2	1	2	1	2	1	1	0	10
Cardenas *et al*[42]	2	2	2	1	2	1	1	1	12
Süoglu *et al*[43]	1	1	1	1	2	1	0	0	7

[1]Assessed with the adapted Methodological Index for Non-Randomized Studies (MINORS)[25]; [2]Clearly stated aim (0,1,2 points); [3]Inclusion of consecutive patients and response rate (0,1,2); [4]Prospective collection of data (0,1,2); [5]Inclusion of employment measure (0,1,2); [6]Unbiased assessment of study end points (0 or 2); [7]Follow-up time appropriate (0,1,2); [8]Loss to follow-up (0,1,2); [9]Prospective calculation of the study size (0 or 1); [10]Total: minimum equals 0; maximum equals 15 points.

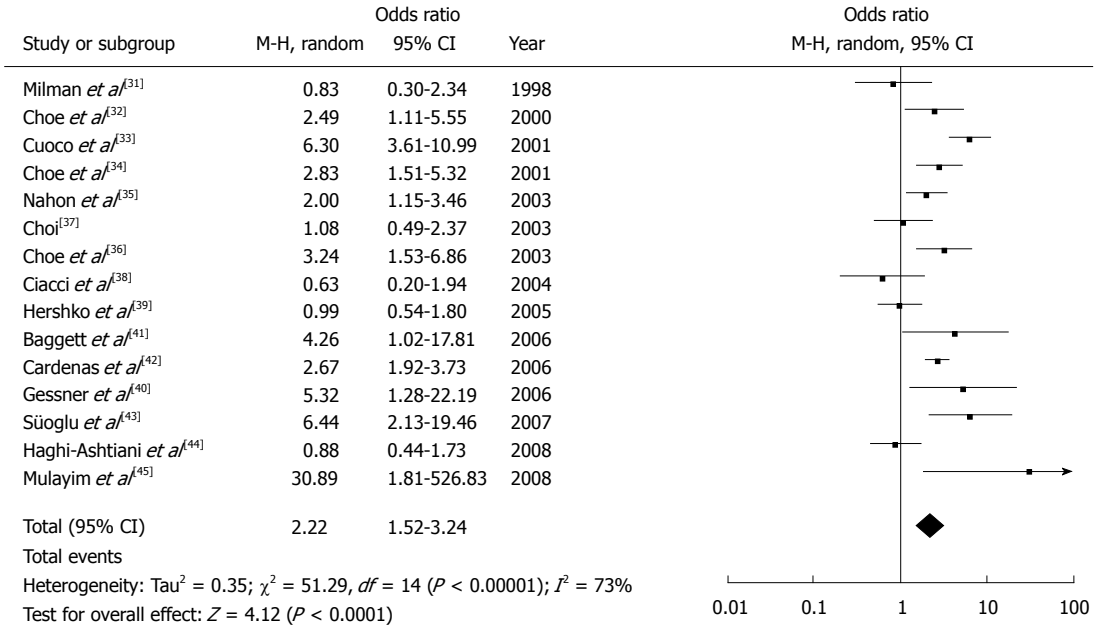

Figure 2 Forest plot of the observational studies. M-H, Random: Mantel-Haenszel heterogeneity random effects model. Horizontal lines = 95% CI. The rectangles represent the point estimates of the study and the size of the rectangle represents the weight given to each study in the meta-analysis. The diamond represents the summary estimate; the size of the diamond represents the CIs of the summary estimate.

Table 3 Quality evaluation of the included studies

RCT	Randomization	Blindness	Withdraw and dropout	Total
Choe *et al*[46]	2	2	0	4
Gessner *et al*[40]	2	1	1	4
Chen *et al*[47]	1	1	1	3
Vijayan *et al*[48]	2	2	1	5
Sarker *et al*[49]	2	0	1	3

SF concentration differences between the intervention and control groups after *H. pylori* eradication therapy. We pooled summary estimates to demonstrate the treatment effect and underlying connection between *H. pylori* and IDA. The results showed that *H. pylori* eradication therapy can improve IDA. Four RCTs compared the increase in HB levels and 5 in SF concentrations achieved with *H. pylori* eradication (plus iron) treatment and with iron administration alone in patients with IDA, and found a greater effect in the eradication group (WMD of HB: 4.06 g/L, 95% CI: -2.57-10.69, $P = 0.01$; WMD of SF: 9.47 μg/L; 95% CI: -0.50-19.43, $P < 0.0001$, Figure 3).

Subgroup analysis

Subgroup analysis was performed to investigate the source of heterogeneity and detect the influential factors that could impact the summary estimates. The methodological

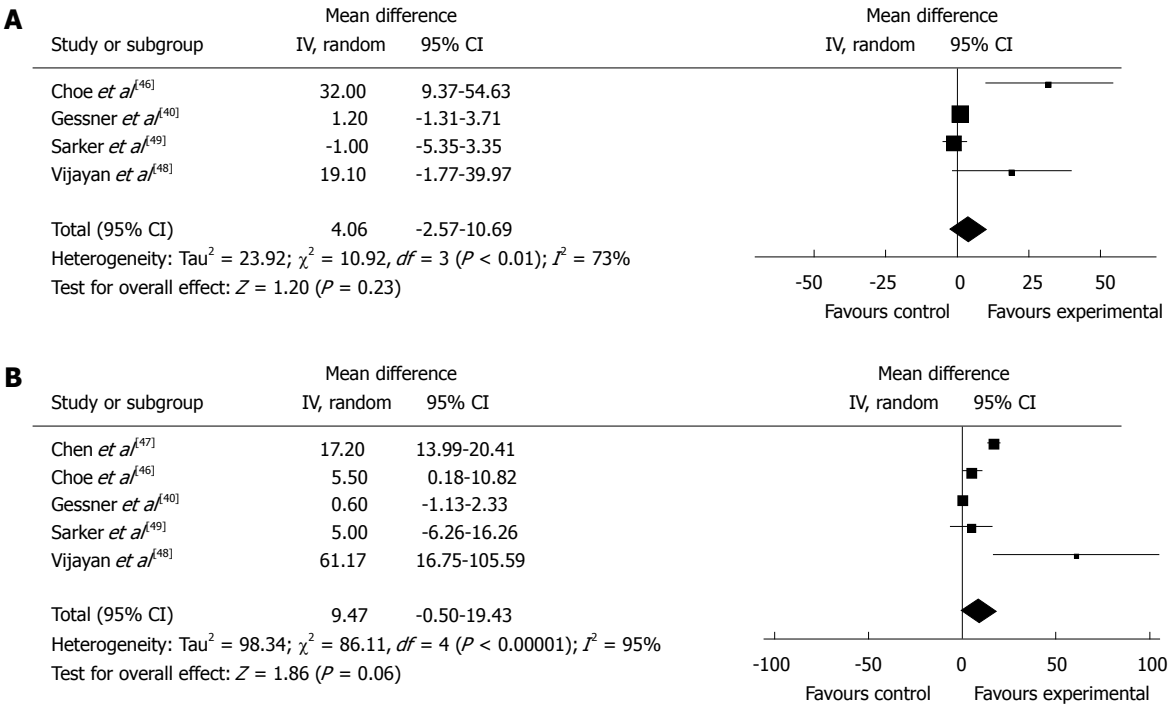

Figure 3 The treatment effect and underlying connection between *H. pylori* and IDA. A: Weighted mean difference (WMD) forest plots of HB (g/L) involved in the meta-analysis; B: Forest plots of studies estimate changes in SF (μg/L) level. IV, Random: Inverse variance heterogeneity random effects model. Horizontal lines = 95% CI. The size of the data marker corresponds to the weight of that study. The diamond represents the summary estimate. The result favors experimental groups.

and biological heterogeneity of the studies made it possible to explore the summary estimates in many different subgroups.

In observational studies, differences in the sensitivity of the *H. pylori* test methods could partly result in different pooled ORs. The 15 observational studies utilized 3 methods, enzyme-linked immunosorbent assay (ELISA) serum IgG, histological biopsy and UBT, to test for the presence of *H. pylori*. The pooled OR of ELISA serum IgG was lowest (OR = 2.16, 95% CI: 1.49-3.14, $P = 0.10$) and the pooled OR of UBT was highest (OR = 5.88, 95% CI: 2.27-15.23, $P = 0.47$). The pooled ORs were consistent with the sensitivity of these methods.

Subgroup analysis for different age groups revealed a significant difference between children, adolescents and adults in the association between *H. pylori* and IDA. The pooled OR of children younger than 11 years was 4.76 (95% CI: 1.73-13.08, $P = 0.83$). Adolescents yielded a pooled OR of 2.85 (95% CI: 1.68-4.31, $P = 0.89$), while adults had a pooled OR of 1.55 (95% CI: 0.67-3.62, $P < 0.0001$).

In RCTs, factors included in the subgroup analysis were age and therapy of each study. The summary estimate from children was 0.65 g/L (95% CI: -1.52-2.82, $P = 0.39$) for HB changes, significantly different from the pooled estimate of 25.03 g/L (95% CI: 9.69-40.37, $P = 0.41$) from adolescent and adult groups, indicating that adult IDA patients react more strongly to *H. pylori* eradication therapy. The WMD for SF changes was 0.70 μg/L (95% CI: -1.01-2.41, $P = 0.45$) in children while the WMD was 14.79 μg/L (95% CI: 2.53-27.05, $P = 0.0001$) in adolescent and adult patients.

To examine the method of therapy, we separated the studies into a bismuth triple therapy group and a proton pump inhibitor (PPI) triple therapy group. Bismuth triple therapy showed an obvious advantage (WMD of SF = 11.55 μg/L, 95% CI: 0.09- 23.01, $P = 0.0002$) over PPI triple therapy (WMD of SF = 7.15 μg/L, 95% CI: -6.45-20.75, $P = 0.002$), particularly when used together with oral ferrous sulfate for *H. pylori* patients with IDA.

We analyzed the association between *H. pylori* and IDA in developed areas and less developed areas, large sample subgroups and small sample subgroups, and did not find any significant difference between those parameters. Table 4 shows the summary estimates of subgroups of both RCTs and observational studies.

Sensitivity analysis

We chose to change the weights of every observational study involved so as to detect the stability of this meta-analysis. We then reanalyzed the data using different statistical methods. The pooled OR using a fixed effects model was 2.34 (95% CI: 1.97-2.78), which is not a significant change from the original random effects model ($P = 0.74$).

Publication bias

Visual inspection of the Begg's funnel plot revealed asymmetry ($P < 0.01$). This raises the possibility of publication bias, so we undertook a sensitivity analysis using the trim and fill method. This method conservatively imputes hypothetical negative unpublished studies

Table 4 Summary of subgroup analyses of both observational studies and experimental studies

	Subjects (*n*)		95% CI	Heterogeneity (χ^2)	*P*[1]
Observational studies		Odds ratio (OR)			
H. pylori test methods					
ELISA serum IgG	12372	2.16	1.49-3.14	9.27	0.10
Histological biopsy	906	2.17	0.90-5.26	28.85	< 0.0001
UBT	1475	5.88	2.27-15.23	1.53	0.47
Age					
Child	1358	4.76	1.73-13.08	0.05	0.83
Adolescent	1972	2.85	1.68-4.31	0.22	0.89
Adult	3101	1.55	0.67-3.62	28.41	< 0.0001
Randomized controlled trials[2]		Weighted mean difference (WMD)			
Hemoglobin (g/L)					
Age					
Child	300	0.65	-1.52-2.82	0.74	0.39
Adolescent and adult	35	25.03	9.69-40.37	0.67	0.41
Therapy					
PPI	322	0.95	-2.98-4.87	3.71	0.16
Bismuth	13	32.00	9.37-54.63	-	-
H. pylori test methods					
Histological biopsy	223	7.03	-9.41-23.47	2.79	0.10
UBT	35	25.03	9.69-40.37	0.67	0.41
Serum ferritin (μg/L)					
Age					
Child	300	0.70	-1.01-2.41	0.57	0.45
Adolescent and adult	121	14.79	2.53-27.05	17.91	0.0001
Therapy					
PPI	322	7.15	-6.45-20.75	7.68	0.002
Bismuth	99	11.55	0.09-23.01	13.61	0.0002

[1]*P*-value tested for heterogeneity of subgroups; [2]Data were derived from hemoglobin changes and serum ferritin changes.

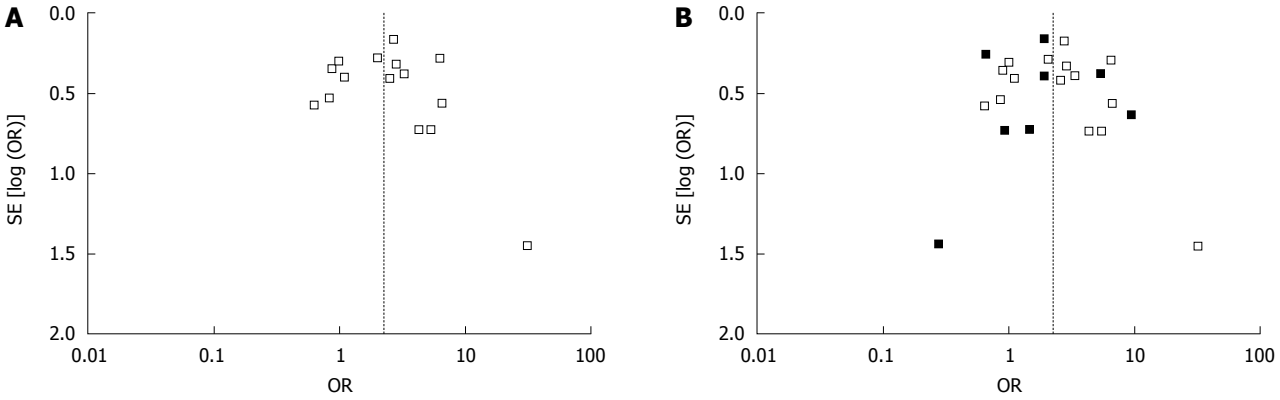

Figure 4 Funnel plots without (A) and with (B) trim and fill. The pseudo 95% CI is computed as part of the analysis that produces the funnel plot, and corresponds to the expected 95% CI for a given standard error (SE). OR: Odds ratio.

to mirror the positive studies that cause funnel plot asymmetry. The adjusted summary OR is based on the eventually filled funnel plot (4.32, 95% CI: 3.00-5.66, $P < 0.001$), which continued to show a statistically significant association between *H. pylori* and IDA (Figure 4).

DISCUSSION

Our results from a meta-analysis of 15 observational epidemiological studies revealed a correlation between *H. pylori* and IDA (OR, 2.22; 95% CI: 1.52-3.24, $P < 0.0001$), although some studies reported only a slight association. In addition, In RCTs, eradication of *H. pylori* can improve HB and SF levels but not significantly (WMD of HB: 4.06 g/L, 95% CI: -2.57-10.69, $P = 0.01$; WMD of SF: 9.47 μg/L, 95% CI: -0.50-19.43, $P < 0.0001$).

Dufour *et al*[50] first reported that *H. pylori* eradication had a positive effect on sideropenic refractory anemia, indicating a possible underlying association between *H. pylori* and IDA. A large population-based study from the USA reported that *H. pylori* infection was an independent risk factor for IDA in 7462 children, adolescents, and adults[42]. This research reported that *H. pylori* infection was associated with an increased risk of IDA (OR, 2.6; 95% CI: 1.5-4.6). Compared to former studies, our meta-analysis was a detailed and comprehensive investigation

procedure. It included RCTs of highly detailed power and large-scale observational epidemiology studies.

It is reported that *H. pylori* infection is observed in over 50% people in the world with peaks of 70%-90% for some countries. Moreover, IDA affects 2 billion people in the world. When 2 diseases have such a high prevalence in the population they may appear to be associated with each other. Recent studies regarding the role of *H. pylori* infection in IDA are controversial. However, whether eradication of *H. pylori* prevents IDA has been widely debated. This meta-analysis was performed to clarify this issue: whether iron deficiency could specifically be related to *H. pylori* infection. The observational studies in our meta-analysis prove the association between *H. pylori* and IDA. In fact, in the RCTs of our meta-analysis, after iron replacement, HB and SF were not different between the groups with or without *H. pylori* eradication. However, these data should be interpreted with caution because of the marked heterogeneity among studies. We carefully performed subgroup analysis, and found age and therapies had an impact on the increase in the levels of HB and SF. These results should be investigated further in the future. Larger scale RCTs should be recommended to test the results of our meta-analysis.

As with all meta-analyses, the results we obtained could be impacted by 3 factors: heterogeneity within the studies involved, bias (including selection bias[51,52] and detection bias[53,54]), and publication bias[55-57]. The generation of heterogeneity could occur by virtue of the methodological and biological heterogeneity of the studies analyzed, such as differences in diagnostic methods, the population under study, the sample size, and language of publication. Each of the subgroups described above contributed partly to the heterogeneity of the observational studies.

We assessed the included studies with caution. For observational studies, the MINORS quality score ranged from 6 to 14 points. In RCTs, quality of all the studies were evaluated by the Jadad sore. All of the studies included had a score greater than 3. Our test for heterogeneity was significant, and hence we utilized a random effects model that accounted for inter-study variation. Compared with the fixed effects model, the random effects model evenly distributes weight among studies, minimizing the impact of heterogeneity[58].

We used age as one subset determinant for the pooled estimates. A significant association between *H. pylori* and IDA was found in children younger than 11 years. The common causes of IDA, such as colonic carcinoma, gastrectomy or menstruation, were usually absent in children. Thus, *H. pylori* infection can be treated as the only indicator for refractory IDA in children, and *H. pylori* infection should be considered first in children with IDA[59-61]. The same scenario occurred in adolescents, as adolescents are particularly susceptible to ID. Because of the requirement for a large amount of iron to sustain their growth, dietary deficiency, and menstrual blood loss, girls should be more strongly affected by *H. pylori* infection[10,34,62]. In adults, no such strong association was found. The possible explanation for this phenomenon is that *H. pylori* plays a smaller part in the etiology of IDA in adults[1,5,63]. In RCT subgroup analysis, eradication therapy for *H. pylori* did not demonstrate the same curative effect on IDA in children as in adults. This may arise from the special characteristics of children (quickly growing blood volume and large requirement)[59-61].

Different diagnostic methods for *H. pylori* contribute to the variation of the pooled estimates because of their different sensitivities. The pooled OR value increased with the sensitivity of the diagnostic method. Therefore, UBT, which has the highest sensitivity of the 3 tests used[64-66], obtained the highest pooled OR, while ELISA serum IgG tests yielded the lowest pooled OR. Recent guidelines have indicated that UBT is regarded as a gold standard diagnostic method and the most reliable nonendoscopic test for the existence of *H. pylori*[67].

The way in which RCTs chose to eradicate *H. pylori* can make a difference in pooled analyses. Bismuth-based triple therapy indicated a much better response to iron intake than PPI-based triple therapy. The work done by McColl and Hutchinson may explain this phenomenon[68,69]. It was reported that PPI therapy lowers the concentration of vitamin C in gastric juice and reduces the bioavailability of ingested vitamin C thus resulting in low absorption of nonheme iron. It may also retard the clinical response to iron supplementation. Vitamin C, as an essential factor in alimentary iron absorption, not only converts ferric iron to the ferrous form, which maintains solubility at the alkaline pH of the duodenum, but also chelates with ferric chloride which is also stable at a pH > 3. PPI can also reduce the absorption of vitamin B_{12}, a significant factor in iron absorption, probably by inhibiting intragastric proteolysis.

Publication bias was tested by Begg's test and illustrated by funnel plots. The results indicated the existence of publication bias. The funnel plot showed that there were some missing small sample studies. Therefore, meta-analysis would underestimate the association between IDA and *H. pylori*. The "trim and fill" method helped to resolve this problem by imputing the hypothetical studies symmetrically and reassessing the pooled estimates as a sensitivity analysis. The filled funnel plot showed a strong association between IDA and *H. pylori*. Possible sources of asymmetry in funnel plots were explored: variations in sample size, etc, could contribute to publication bias.

Sensitivity analysis was performed in several ways to test concordance of the results by changing the statistical methods used. The sensitivity analyses that were performed did not materially change the results, increasing the confidence that can be placed in these results when applying the conclusion in practice.

Our study has limitations. We have excluded trials that studied the relationship between *H. pylori* and iron deficiency. However, these studies have been reviewed elsewhere[70]. Furthermore, in the experimental studies,

eradication treatment without ferrous sulfate but with placebo groups were excluded because the number of studies was not adequate. This kind of design can illustrate the role that *H. pylori* plays in IDA in a better way, and we expect further investigations will take that design into consideration. Lastly, results were markedly heterogeneous for all comparisons. These data should be interpreted with caution because of the marked heterogeneity among studies.

In conclusion, our meta-analysis of 15 observational studies demonstrated an association between *H. pylori* and IDA. In addition, the meta-analysis of RCTs showed that eradication of *H. pylori* can improve HB and SF levels, though not significantly. From the analysis, we also concluded that IDA could not specifically be related to *H. pylori* infection. We do not recommend a strategy of population-based screening and treatment for *H. pylori* infection to prevent IDA. This concept should be discussed in the future. UBT is the most reliable nonendoscopic test for the existence of. Bismuth-based triple therapy has a better response to increase HB and SF levels than PPI-based triple therapy. There are no significant differences between less developed areas and developed areas in the association between *H. pylori* infection and IDA.

COMMENTS

Background

Both *Helicobacter pylori* (*H. pylori*) and iron deficiency anemia (IDA) have high prevalence worldwide. The relationship between these 2 remains controversial. Recent guidelines for *H. pylori* and IDA both focus on the role *H. pylori* plays in the process of IDA.

Research frontiers

The interaction between *H. pylori* and IDA is a current 'hot topic'. *H. pylori* infection impairs iron absorption causing a considerable decrease in the concentration of gastric juice ascorbic acid (vitamin C) that is the best promoter of nonheme iron absorption. It thus causes the decline of hemoglobin in red blood cells and directly leads to anemia. The gastric colonization by *H. pylori* increases lactoferrin uptake from neutrophils and increases iron demand.

Innovations and breakthroughs

To the best of the authors' knowledge, this is the first published meta-analysis assessing the association between *H. pylori* infection and IDA (evaluating children, adolescents and adults) and assessing the effect of *H. pylori* eradication on hemoglobin (HB) and serum ferritin (SF) levels.

Applications

The research showed an association between *H. pylori* and IDA. Eradication of *H. pylori* can improve HB and SF levels but not significantly. The authors do not recommend a strategy of population-based screening and treatment for *H. pylori* infection to prevent IDA.

Peer review

This is a good and interesting meta-analysis.

REFERENCES

1 World Health Organization, The United Nations Children's Fund, United Nations University. Iron deficiency anaemia: assessment, prevention, and control. Available from: URL: http://www.who.int/nut/documents/ida_assessment_prevention_control.pdf, accessed 27 July, 2004
2 **Zimmermann MB**, Hurrell RF. Nutritional iron deficiency. *Lancet* 2007; **370**: 511-520
3 **Clark SF**. Iron deficiency anemia: diagnosis and management. *Curr Opin Gastroenterol* 2009; **25**: 122-128
4 **Jolobe O**. Guidelines for the management of iron deficiency anaemia. *Gut* 2001; **49**: 158
5 World Health Organization, United Nations University, The United Nations Children's Fund. Iron deficiency anaemia. Assessment, prevention and control:A guide for programme managers. Available from: URL: http://whqlibdoc.who.int/hq/2001/WHO_NHD_01.3.pdf, accessed 2001
6 **Ramakrishnan U**. Prevalence of micronutrient malnutrition worldwide. *Nutr Rev* 2002; **60**: S46-S52
7 World Health Organization. Micronutrient deficiencies—Iron deficiency anaemia. Available from: URL: http://www.who.int/nutrition/topics/ida/en/index.html, accessed 2001
8 **Weiss G**, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; **352**: 1011-1023
9 **Annibale B**, Marignani M, Monarca B, Antonelli G, Marcheggiano A, Martino G, Mandelli F, Caprilli R, Delle Fave G. Reversal of iron deficiency anemia after Helicobacter pylori eradication in patients with asymptomatic gastritis. *Ann Intern Med* 1999; **131**: 668-672
10 **Gasche C**, Lomer MC, Cavill I, Weiss G. Iron, anaemia, and inflammatory bowel diseases. *Gut* 2004; **53**: 1190-1197
11 **DuBois S**, Kearney DJ. Iron-deficiency anemia and Helicobacter pylori infection: a review of the evidence. *Am J Gastroenterol* 2005; **100**: 453-459
12 **Bini EJ**. Helicobacter pylori and iron deficiency anemia: guilty as charged? *Am J Med* 2001; **111**: 495-497
13 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781
14 **Howden CW**, Hunt RH. Guidelines for the management of Helicobacter pylori infection. Ad Hoc Committee on Practice Parameters of the American College of Gastroenterology. *Am J Gastroenterol* 1998; **93**: 2330-2338
15 World Gastroenterology Organisation. WGO Practice Guideline: Helicobacter pylori in Developing Countries. Available from: URL: http://www.worldgastroenterology.org/assets/downloads/en/pdf/guidelines/11_helicobacter_pylori_developing_countries_en.pdf, accessed 2005
16 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186
17 **Huang JQ**, Sridhar S, Hunt RH. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. *Lancet* 2002; **359**: 14-22
18 **Wong BC**, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004; **291**: 187-194
19 **Jaakkimainen RL**, Boyle E, Tudiver F. Is Helicobacter pylori associated with non-ulcer dyspepsia and will eradication improve symptoms? A meta-analysis. *BMJ* 1999; **319**: 1040-1044
20 **Gasbarrini A**, Franceschi F, Armuzzi A, Ojetti V, Candelli M, Torre ES, De Lorenzo A, Anti M, Pretolani S, Gasbarrini G. Extradigestive manifestations of Helicobacter pylori gastric infection. *Gut* 1999; **45** Suppl 1: I9-I12
21 **Peterson WL**, Fendrick AM, Cave DR, Peura DA, Garabedian-Ruffalo SM, Laine L. Helicobacter pylori-related disease: guidelines for testing and treatment. *Arch Intern Med* 2000; **160**: 1285-1291
22 **Leontiadis GI**, Sharma VK, Howden CW. Non-gastrointestinal tract associations of Helicobacter pylori infection. *Arch Intern Med* 1999; **159**: 925-940
23 **Moher D**, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of Reporting of Meta-analyses. *Lancet* 1999; **354**: 1896-1900

24 **Stroup DF**, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008-2012

25 **Slim K**, Nini E, Forestier D, Kwiatkowski F, Panis Y, Chipponi J. Methodological index for non-randomized studies (minors): development and validation of a new instrument. *ANZ J Surg* 2003; **73**: 712-716

26 **Jadad AR**, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996; **17**: 1-12

27 The Cochrane Collaboration. Cochrane Handbook for Systematic Reviews of Interventions Version 5.0.0[updated September 2008]. Available from: URL: http://www.cochrane-handbook.org/. accessed 7 November, 2008

28 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188

29 **Higgins JP**, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**: 1539-1558

30 **Begg C**, Cho M, Eastwood S, Horton R, Moher D, Olkin I, Pitkin R, Rennie D, Schulz KF, Simel D, Stroup DF. Improving the quality of reporting of randomized controlled trials. The CONSORT statement. *JAMA* 1996; **276**: 637-639

31 **Milman N**, Rosenstock S, Andersen L, Jørgensen T, Bonnevie O. Serum ferritin, hemoglobin, and Helicobacter pylori infection: a seroepidemiologic survey comprising 2794 Danish adults. *Gastroenterology* 1998; **115**: 268-274

32 **Choe YH**, Kim SK, Hong YC. Helicobacter pylori infection with iron deficiency anaemia and subnormal growth at puberty. *Arch Dis Child* 2000; **82**: 136-140

33 **Cuoco L**, Cammarota G, Jorizzo RA, Santarelli L, Cianci R, Montalto M, Gasbarrini A, Gasbarrini G. Link between Helicobacter pylori infection and iron-deficiency anaemia in patients with coeliac disease. *Scand J Gastroenterol* 2001; **36**: 1284-1288

34 **Choe YH**, Kwon YS, Jung MK, Kang SK, Hwang TS, Hong YC. Helicobacter pylori-associated iron-deficiency anemia in adolescent female athletes. *J Pediatr* 2001; **139**: 100-104

35 **Nahon S**, Lahmek P, Massard J, Lesgourgues B, Mariaud de Serre N, Traissac L, Bodiguel V, Adotti F, Delas N. Helicobacter pylori-associated chronic gastritis and unexplained iron deficiency anemia: a reliable association? *Helicobacter* 2003; **8**: 573-577

36 **Choe YH**, Kim SK, Hong YC. The relationship between Helicobacter pylori infection and iron deficiency: seroprevalence study in 937 pubescent children. *Arch Dis Child* 2003; **88**: 178

37 **Choi JW**. Does Helicobacter pylori infection relate to iron deficiency anaemia in prepubescent children under 12 years of age? *Acta Paediatr* 2003; **92**: 970-972

38 **Ciacci C**, Sabbatini F, Cavallaro R, Castiglione F, Di Bella S, Iovino P, Palumbo A, Tortora R, Amoruso D, Mazzacca G. Helicobacter pylori impairs iron absorption in infected individuals. *Dig Liver Dis* 2004; **36**: 455-460

39 **Hershko C**, Hoffbrand AV, Keret D, Souroujon M, Maschler I, Monselise Y, Lahad A. Role of autoimmune gastritis, Helicobacter pylori and celiac disease in refractory or unexplained iron deficiency anemia. *Haematologica* 2005; **90**: 585-595

40 **Gessner BD**, Baggett HC, Muth PT, Dunaway E, Gold BD, Feng Z, Parkinson AJ. A controlled, household-randomized, open-label trial of the effect that treatment of Helicobacter pylori infection has on iron deficiency in children in rural Alaska. *J Infect Dis* 2006; **193**: 537-546

41 **Baggett HC**, Parkinson AJ, Muth PT, Gold BD, Gessner BD. Endemic iron deficiency associated with Helicobacter pylori infection among school-aged children in Alaska. *Pediatrics* 2006; **117**: e396-e404

42 **Cardenas VM**, Mulla ZD, Ortiz M, Graham DY. Iron deficiency and Helicobacter pylori infection in the United States. *Am J Epidemiol* 2006; **163**: 127-134

43 **Süoglu OD**, Gökçe S, Saglam AT, Sökücü S, Saner G. Association of Helicobacter pylori infection with gastroduodenal disease, epidemiologic factors and iron-deficiency anemia in Turkish children undergoing endoscopy, and impact on growth. *Pediatr Int* 2007; **49**: 858-863

44 **Haghi-Ashtiani MT**, Monajemzadeh M, Motamed F, Mahjoub F, Sharifan M, Shahsiah R, Kashef N. Anemia in children with and without Helicobacter pylori infection. *Arch Med Res* 2008; **39**: 536-540

45 **Mulayim B**, Celik NY, Yanik FF. Helicobacter pylori infection detected by 14C-urea breath test is associated with iron deficiency anemia in pregnant women. *J Obstet Gynaecol Res* 2008; **34**: 980-985

46 **Choe YH**, Kim SK, Son BK, Lee DH, Hong YC, Pai SH. Randomized placebo-controlled trial of Helicobacter pylori eradication for iron-deficiency anemia in preadolescent children and adolescents. *Helicobacter* 1999; **4**: 135-139

47 **Chen LH**, Luo HS. Effects of H. pylori therapy on erythrocytic and iron parameters in iron deficiency anemia patients with H. pylori-positive chronic gastristis. *World J Gastroenterol* 2007; **13**: 5380-5383

48 **Vijayan G**, Sundaram RC, Bobby Z, Hamide A, Selvaraj N, Dasse NR. Increased plasma malondialdehyde and fructosamine in anemic H. pylori infected patients: effect of treatment. *World J Gastroenterol* 2007; **13**: 796-800

49 **Sarker SA**, Mahmud H, Davidsson L, Alam NH, Ahmed T, Alam N, Salam MA, Beglinger C, Gyr N, Fuchs GJ. Causal relationship of Helicobacter pylori with iron-deficiency anemia or failure of iron supplementation in children. *Gastroenterology* 2008; **135**: 1534-1542

50 **Dufour C**, Brisigotti M, Fabretti G, Luxardo P, Mori PG, Barabino A. Helicobacter pylori gastric infection and sideropenic refractory anemia. *J Pediatr Gastroenterol Nutr* 1993; **17**: 225-227

51 **Kunz R**, Vist G, Oxman AD. Randomisation to protect against selection bias in healthcare trials. *Cochrane Database Syst Rev* 2007; MR000012

52 **Guyatt GH**, Oxman AD, Kunz R, Vist GE, Falck-Ytter Y, Schünemann HJ. What is "quality of evidence" and why is it important to clinicians? *BMJ* 2008; **336**: 995-998

53 **Schulz KF**, Chalmers I, Hayes RJ, Altman DG. Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. *JAMA* 1995; **273**: 408-412

54 **Karlowski TR**, Chalmers TC, Frenkel LD, Kapikian AZ, Lewis TL, Lynch JM. Ascorbic acid for the common cold. A prophylactic and therapeutic trial. *JAMA* 1975; **231**: 1038-1042

55 **Easterbrook PJ**, Berlin JA, Gopalan R, Matthews DR. Publication bias in clinical research. *Lancet* 1991; **337**: 867-872

56 **Dickersin K**. The existence of publication bias and risk factors for its occurrence. *JAMA* 1990; **263**: 1385-1389

57 **Sutton AJ**, Duval SJ, Tweedie RL, Abrams KR, Jones DR. Empirical assessment of effect of publication bias on meta-analyses. *BMJ* 2000; **320**: 1574-1577

58 **Poole C**, Greenland S. Random-effects meta-analyses are not always conservative. *Am J Epidemiol* 1999; **150**: 469-475

59 **Oski FA**. Iron deficiency in infancy and childhood. *N Engl J Med* 1993; **329**: 190-193

60 **Kurekci AE**, Atay AA, Sarici SU, Yesilkaya E, Senses Z, Okutan V, Ozcan O. Is there a relationship between childhood Helicobacter pylori infection and iron deficiency anemia? *J Trop Pediatr* 2005; **51**: 166-169

61 **Russo-Mancuso G**, Branciforte F, Licciardello M, La Spina M. Iron deficiency anemia as the only sign of infection with Helicobacter pylori: a report of 9 pediatric cases. *Int J Hematol*

2003; **78**: 429-431
62 **Hallberg L**, Hultén L, Lindstedt G, Lundberg PA, Mark A, Purens J, Svanberg B, Swolin B. Prevalence of iron deficiency in Swedish adolescents. *Pediatr Res* 1993; **34**: 680-687
63 **Yip R**, Johnson C, Dallman PR. Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. *Am J Clin Nutr* 1984; **39**: 427-436
64 **Thijs JC**, van Zwet AA, Thijs WJ, Oey HB, Karrenbeld A, Stellaard F, Luijt DS, Meyer BC, Kleibeuker JH. Diagnostic tests for Helicobacter pylori: a prospective evaluation of their accuracy, without selecting a single test as the gold standard. *Am J Gastroenterol* 1996; **91**: 2125-2129
65 **Atherton JC**, Spiller RC. The urea breath test for Helicobacter pylori. *Gut* 1994; **35**: 723-725
66 **Logan RP**, Polson RJ, Misiewicz JJ, Rao G, Karim NQ, Newell D, Johnson P, Wadsworth J, Walker MM, Baron JH. Simplified single sample 13Carbon urea breath test for Helicobacter pylori: comparison with histology, culture, and ELISA serology. *Gut* 1991; **32**: 1461-1464
67 **Chey WD**, Wong BC. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. *Am J Gastroenterol* 2007; **102**: 1808-1825
68 **McColl KE**. Effect of proton pump inhibitors on vitamins and iron. *Am J Gastroenterol* 2009; **104** Suppl 2: S5-S9
69 **Hutchinson C**, Geissler CA, Powell JJ, Bomford A. Proton pump inhibitors suppress absorption of dietary non-haem iron in hereditary haemochromatosis. *Gut* 2007; **56**: 1291-1295
70 **Muhsen K**, Cohen D. Helicobacter pylori infection and iron stores: a systematic review and meta-analysis. *Helicobacter* 2008; **13**: 323-340

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