

# Helicobacter pylori infection and micronutrient deficiency in pregnant women: a systematic review and meta-analysis

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## ABSTRACT

**Background** Over the last few years, epidemiological studies have shown that infection with *Helicobacter pylori* has a major effect on micronutrient deficiency as well as on adverse pregnancy outcomes. Importantly, there are gaps in understanding the linkage of *H. pylori* infection with micronutrient deficiency in pregnant women.

**Objective** We conducted a systematic review and meta-analysis to estimate the association between *H. pylori* infection and micronutrient deficiencies in pregnant women.

**Methods** A systematic literature search was conducted for relevant articles using PubMed, Web of Science, and Scopus database from inception to March 2020. The OR with 95% CIs was determined by meta-analysis of data extracted from the selected studies.

**Results** From 2384 primary articles, 6 studies were selected for systematic reviews and 4 studies distinctively (with 1274 participants: 553 cases and 721 controls) were selected for meta-analysis. The meta-analysed fixed effect model estimated the odds of having *H. pylori* infection was not significantly higher among pregnant women with micronutrient deficiencies than those without deficiencies (OR=1.12, 95% CI 0.88 to 1.42, p=0.37). In the subgroup analysis, no correlation was found between *H. pylori* infection and vitamin B<sub>12</sub> (OR=0.74, 95% CI 0.45 to 1.21, p=0.22), folate (OR=1.07, 95% CI 0.73 to 1.58, p=0.73), and ferritin (OR=0.81, 95% CI 0.51 to 1.31, p=0.4). However, a positive correlation was found between iron-deficiency anaemia (IDA) and *H. pylori* infection (OR=16.23, 95% CI 4.19 to 62.93, p<0.0001) during pregnancy.

**Conclusion** *H. pylori* infection is associated with increased risk of IDA but not with deficiency of other micronutrients in pregnancy.

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## INTRODUCTION

The balanced store of nutrient during pregnancy plays a crucial role in preserving the maternal well-being, as well as for the optimal growth and development of the fetus. Macronutrients (carbohydrate, protein and fat)

serve as building blocks and provide energy whereas micronutrients, including vitamins and minerals, are indispensable for metabolism, immune function, and brain development.<sup>1 2</sup> In 2015, WHO reported that globally, 32 million women became anaemic during pregnancy which was 15%–20% of total pregnant women,<sup>3</sup> noticeably around 19 million were reported to be vitamin A deficient, and millions suffer from insufficient iron, folate, zinc or iodine stores. Moreover, about 20 million babies with low birth weight (LBW) are born weighing less than 2500 g at birth.<sup>4</sup> An estimated 15 million babies are born premature, and many more are born with other complications which increase their risk of morbidity and mortality during childhood.<sup>4</sup> It has been shown that poor micronutrient status is highly correlated with adverse pregnancy outcomes and subsequent childhood health.<sup>3</sup> Several micronutrient supplementations programmes have been implemented to improve the micronutrient status of mother. A review by Alison *et al*<sup>3</sup> reported that multiple micronutrient supplementations reduced the risk of poor pregnancy outcomes, while other studies found that maternal health was unchanged after supplementation of folic acid, iron, vitamin B<sub>12</sub> and other micronutrients.<sup>5–7</sup> In addition to poor health conditions, inadequate dietary intake, low socioeconomic condition and lack of sanitary knowledge, the prevalence of acute and chronic diseases caused by *H. pylori* is also responsible for the nutrient deficiency during pregnancy.<sup>8</sup>

*H. pylori*, a curved gram-negative ciliated bacillus, is responsible for stomach infection in 44.3% of the global population.<sup>9</sup> *H. pylori* infection is highly prevalent in pregnant women, as pregnancy itself is a risk factor for the infection.<sup>10</sup> Hormonal changes and

immunological alterations that ensure maternal tolerance towards the fetus can trigger dormant *H. pylori* into infectious during pregnancy.<sup>11</sup> This pathogen further alters gastric physiology with a chronic inflammatory response of the gastric mucosa. It also produces phospholipase A2 and C, and glycosulfatase enzymes that play vital roles in damaging the gastric mucosal layer.<sup>12</sup> This eventually gives rise to higher gastric pH,<sup>13</sup> which can affect nutrient and drug absorption.<sup>14 15</sup> Moreover, *H. pylori* is associated with severe nausea and vomiting or hyperemesis gravidarum in pregnancy period and this could result in malnutrition as well, especially the loss of water-soluble vitamins.<sup>16</sup> Prior research exhibited that *H. pylori* infection has a role in the pathogenesis of various pregnancy related disorders including iron-deficiency anaemia (IDA), fetal malformations and fetal growth restriction.<sup>17 18</sup> IDA may coexist with vitamin B<sub>12</sub> and folate deficiency which are associated with poor birth outcomes such as premature birth, intrauterine growth retardation, infertility or recurrent spontaneous abortion.<sup>19</sup> Besides, it is probable that dietary vitamin B<sub>12</sub> deficiency may be exacerbated due to malabsorption caused by *H. pylori* infection.<sup>15</sup>

Although there are some ambiguous findings, research has not been carried out to address systematically the association between *H. pylori* infection and micronutrients deficiency in pregnant women. Previous systematic reviews have highlighted the association between *H. pylori* infection and negative pregnancy outcomes<sup>20</sup> and the association between *H. pylori* infection and micronutrient deficiencies in the general population.<sup>21</sup> Therefore, our objective was to conduct a systematic review and meta-analysis to investigate whether *H. pylori* infection is linked with reduced concentration of micronutrients among pregnant women, who are highly vulnerable to infection and malnutrition.

## MATERIALS AND METHODS

### Registration of review protocol

The study was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses and Meta-analysis of Observational Studies in Epidemiology guidelines for systematic review and meta-analysis. We registered the protocol prospectively in PROSPERO.

### Literature search

Search strategies were prepared and designated according to the PICOS principle method:

P (population): pregnant OR mother OR pregnancy OR child bearing age OR women.

I (intervention/exposure): *H. pylori* OR *Helicobacter pylori*.

C (comparison): without *H. pylori* infection.

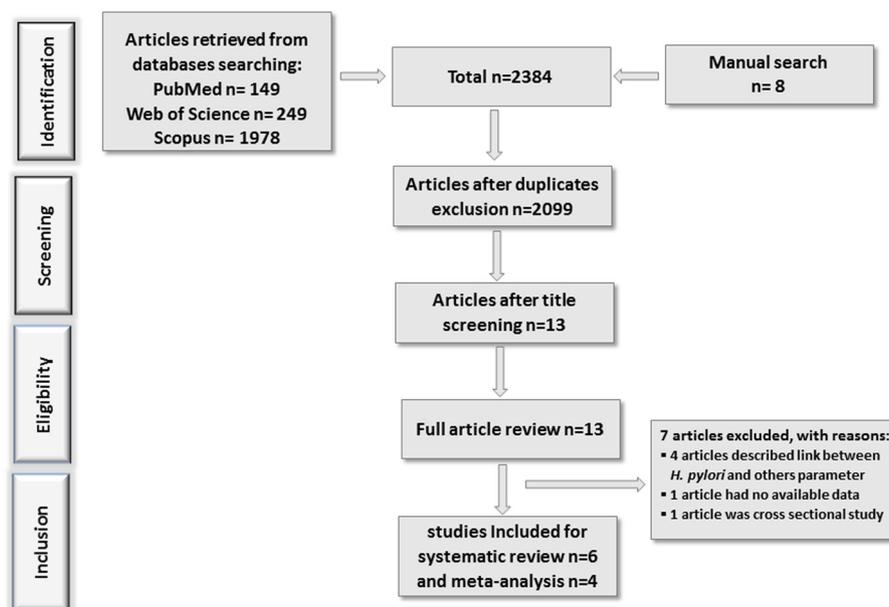
O (outcome): nutrition OR malnutrition OR micronutrients OR nutrients OR undernutrition OR nutritional deficiency OR vitamin OR mineral OR trace element OR iron OR folate OR homocysteine OR zinc OR copper OR Iodine OR manganese OR calcium OR magnesium.

S (study design): no restriction.

Articles were searched using PubMed, Web of Science, and Scopus database from inception to March 2020 without restriction to geography. The Boolean operation (ie, AND, OR) was employed to compile the keywords that were searched in all fields format in the free text writing boxes. Manual searches of the relevant article references were also performed. The search strategies of three databases are given in online supplemental materials.

### Selection criteria

Eligible studies were selected based on the following criteria:



**Figure 1** Flow diagram of study inclusion for systematic review and meta-analysis.

**Table 1** Demographic characteristics of the included study participants

Study (year)	Study design	Location	Gestation (week)		Patients (n)		Age (mean±SD)		BMI (mean±SD)	
			HP (+ve)	HP (-ve)	HP (+ve)	HP (-ve)	HP (+ve)	HP (-ve)	HP (+ve)	HP (-ve)
			(Mean±SD)							
Ali et al (2018) <sup>40</sup>	Case control study	Iraq	34.8±5.5	33.6±5.9	50	50	28.1±6.2	29.1±5.6	NM	NM
Felkner et al (2007) <sup>38</sup>	Case control study	USA	NM	NM	95	147	22.9±4.2	23.5±4.7	24±2.3	24±3.8
Golalipour et al (2012) <sup>37</sup>	Case control study	Iran	40±3.3	41±3.3	35	53	25.3±5.4	25.4±5.1	NM	NM
Mulayim et al (2008) <sup>39</sup>	Case control study	Turkey	30±2.4	30±2.7	72	45	28.5±3.47	30±3.1	27±2.1	24±3.6
Mubarak et al (2014) <sup>36</sup>	Cross-sectional study	Sudan	25.1±2.6	25.1±2.6	125	54	26±6.8	26±6.8	28.8±6.32	28.8±6.32
Ugwuja et al (2010) <sup>35</sup>	Mixed method	Nigeria	21.77±3.14	21.77±3.14	84	265	28.19±4.89	26.7±4.65	29.0±3.89	26.86±4.1

BMI, body mass index; HP(-ve), *Helicobacter pylori* negative; HP(+ve), *Helicobacter pylori* positive; NM, not mentioned.

- ▶ Full text English-language original articles including randomised control trial (RCT), cross-sectional study, case-control study, or cohort-study designs.
- ▶ Articles, reported any micronutrient deficiency (at least one) in pregnant women with *H. pylori* infection.
- ▶ Studies that reported the assessment levels of micronutrients in *H. pylori* positives and *H. pylori* negative patients.

The exclusion criteria were as follows:

- ▶ Review articles, case reports, editorials, guidelines, letter to editors and conference articles.
- ▶ Articles written in languages other than English.
- ▶ Studies in non-human, non-pregnant and animal studies.
- ▶ Studies lacking the standard tests for detection of *H. pylori* infection or those with unusual diagnostic methodology.
- ▶ Articles with incomplete or obscure methodology or results.

### Data extraction

Two authors independently screened and evaluated the article title and abstract of the included studies to categorise the relevant research for full-text assessment. Discrepancies were settled by consensus through discussion with the third author (TJS). Using the search strategy, a total of 2384 unique records were initially found. Through cross-referencing and other resources, 8 additional studies were identified. We excluded the irrelevant and duplicated articles from the pooled search results considering the exclusion and inclusion criteria. After that, authors MNAA and ZNJ screened the title and abstract of 2099 articles. MNAA and ZNJ claimed for 15 and 18 articles for full review, respectively. Among those, 11 articles were common. After discussion and meeting with the author TJS, we were on agreement to select 13 articles for full review. On careful reading and scrutinising, we had no dispute to select 6 articles from those 13 articles. The list of excluded articles is given in online supplemental materials. Finally, we selected four articles for quantitative analysis and additionally two articles (total six) for systematic reviews. The following data were retrieved to present the description of the study:

### Study information

First author, publication year, country, type of study design, total sample size, number of cases, number of controls and *H. pylori* detection method.

### Baseline characteristics

Age, gestational age, location and body mass index (BMI).

### Outcomes

Vitamin and trace elements depletion such as vitamin B<sub>12</sub>, folate, iron, copper, zinc and prevalence of IDA was considered for the effect measure. The most recent and comprehensive report was taken into evaluation, when data were reported from overlapping study samples.

**Table 2** Study results evaluating markers of *H. pylori* infection in patients with micronutrients deficiency

Study (Year)	<i>H. pylori</i> diagnosis		Description of the Indicators	Outcomes	Limitations/strength
	Antibody/targeted molecule	Method			
Ali <i>et al</i> (2018) <sup>40</sup>	Anti-Helicobacter pylori IgA	ELISA	Iron	Significant positive correlation between iron deficiency and <i>H. pylori</i> -positive cases.	Smaller sample size, limited access data, study purpose was highly focused.
Felkner <i>et al</i> (2007) <sup>38</sup>	Anti-Helicobacter pylori IgG	ELISA	Ferritin, folate, vitamin B <sub>12</sub>	No association was found between micronutrients and <i>H. pylori</i> -positive cases.	Larger sample size, well-validated study, sample was collected in just after post partum.
Golalipour <i>et al</i> (2012) <sup>37</sup>	Anti-Helicobacter pylori IgG	ELISA	Ferritin, folate, vitamin B <sub>12</sub>	No association was found between micronutrients and <i>H. pylori</i> -positive cases.	Smaller sample size, sample was collected in just after post partum.
Mulayim <i>et al</i> (2008) <sup>39</sup>	Urease	<sup>14</sup> C-urea breath test	Iron	Significant positive correlation between iron deficiency and <i>H. pylori</i> -positive cases.	Data were not normally distributed when calculated for iron deficiency.
Mubarak <i>et al</i> (2014) <sup>36</sup>	Anti-Helicobacter pylori IgA	ELISA	Ferritin	There is a no link between iron deficiency, anaemia, and thrombocytopenia with <i>H. pylori</i> -positive cases.	Larger sample size, well-validated study, limited biasness
Ugwuja <i>et al</i> (2010) <sup>35</sup>	Anti-Helicobacter pylori IgG	ELISA	Copper, iron and zinc	Trace elements (Cu, Fe, and Zn) are not significantly associated with <i>H. pylori</i> positive cases	Larger sample size, limited access data, highly biased.

### Study quality evaluation

The qualities of the included studies were assessed by using relevant version of the Critical Appraisal Skills Programme (CASP) checklists tools.<sup>22</sup> CASP checklist consists of 3 sections with 11 questions in total. Each section explores the trustworthiness, results and relevance of the studies; section A is for validation of the result, section B is for description of the result, and section C is for implementation of the result. Quality of the all included studies was assessed by two authors (MNAA, ZNJ) independently. In case of any divergences, open discussion with the third author (TJS) was executed and the issues were resolved. All the questions were designed for three answers (Yes, Not and Can't tell). If the answer was clearly described, we marked it "Yes" and for the vice versa we marked it "No". If the answer was ambiguous then we marked it "Can't tell". We excluded two questions (questions 7 and 8), not relevant to our studies. Similar with CASP checklist assessment, we appraised the risk of bias (ROB) of case-control studies through the Newcastle-Ottawa (NOS) Scale.<sup>23</sup> We used a scoring method to assess each of the study. The scoring method mainly consists of three aspects: selection of participant (four points), comparability (two points) and outcome (three points).<sup>24</sup> From a total of 9 points, studies with 7–9 were deemed low ROB, whereas studies with 5–6 and <5 points were considered moderate ROB and high ROB, respectively.<sup>25 26</sup>

### Statistical analysis

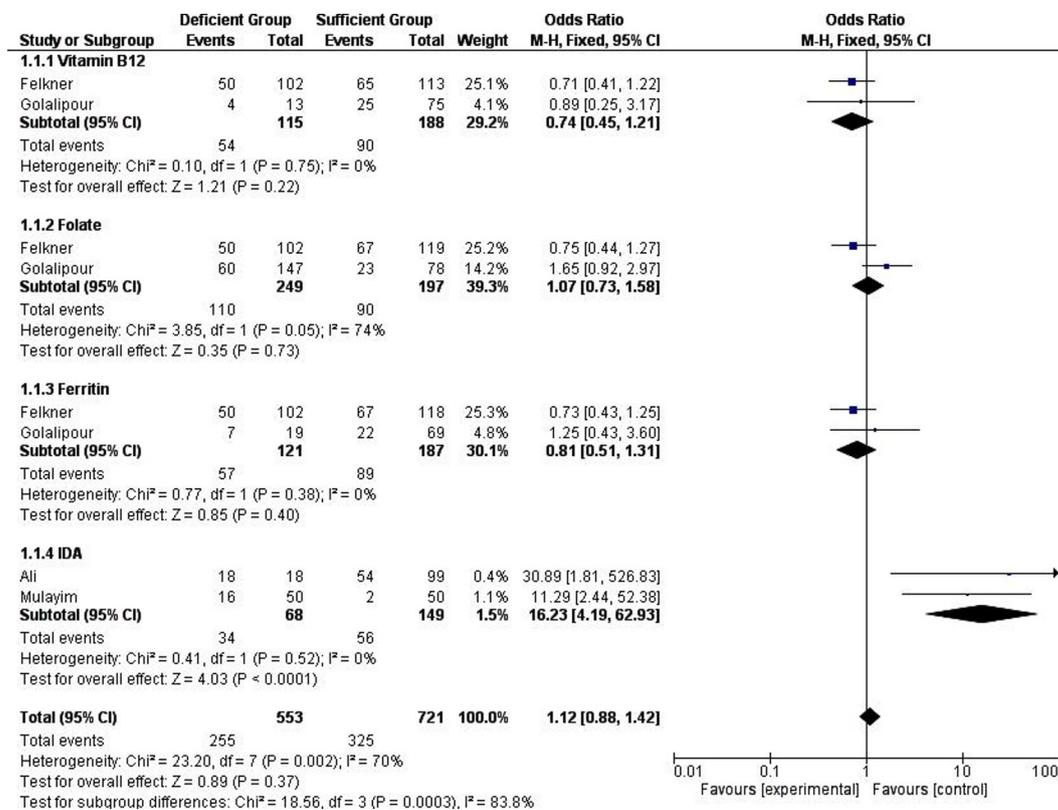
The risk of poor micronutrient status outcome among *H. pylori* infected pregnant women was estimated by the OR with its 95% CIs as the effect size.  $P < 0.05$  was considered

statistically significant. Interstudy heterogeneity was tested by Cochrane Q ( $X^2$ ) test with limiting  $p < 0.10$  level as a significance and estimated by the  $I^2$  statistic with values of 25%, 50%, and 75% indicating low, moderate, and high degrees of heterogeneity, respectively.<sup>27</sup> Funnel plot was constructed to assess publication bias. Along with meta-analysis, subgroup analyses for each of the nutrients (vitamin B<sub>12</sub>, folate, ferritin and iron) were performed and represented by the forest plot. All the statistical analyses were carried out by using Review Manager Software (RevMan V.5.3).

## RESULTS

### Description of the included studies

We recorded 2384 articles according to the previously described search strategy and retrieved article from PubMed (n=149), Web of Sciences (n=249), Scopus (n=1978) and other sources (n=8). In the initial screening, 272 studies were excluded as they were duplicated. Later, 2099 records were excluded due to irrelevant title or abstract, animal trial, or no relevance, leaving 13 studies. After complete reviewing, seven articles were eliminated with reasons: four articles were disqualified because they focused on the association between *H. pylori* seropositivity and gestational weight gain,<sup>28</sup> socioeconomic condition,<sup>29</sup> LBW<sup>30</sup> and diabetic pregnant women.<sup>31</sup> Three articles<sup>32–34</sup> indicated, in result sections that *H. pylori* were the responsible factor for lower iron status in pregnant women, but quantitative data could not be retrieved. Only one study in Nigeria<sup>35</sup> reported that there was no



**Figure 2** Forest plot of the association between *H. pylori* infection and micronutrient deficiency.

significant association between *H. pylori* infection and reduced level of trace element (Cu, Zn, Fe) in pregnant women. However, their published results were presented by mean value; therefore, eligible quantitative data were not found. Another article by Mubarak *et al*<sup>36</sup> concluded that there was no association between *H. pylori* infection and anaemia, iron deficiency, and thrombocytopenia during pregnancy. However, the study was observational in nature, that is, cross sectional. The overall study search and selection was illustrated in figure 1.

Most of the studies had specific exclusion criteria, such as hyperthyroid, gastrointestinal problems, diabetes mellitus, multiple gestation, and prepartum haemorrhage other than *H. pylori* infection. Serum antibody (IgG, IgA or IgM) analysis was performed by using the ELISA method to detect *H. pylori* infection in most of the studies. All four included articles for meta-analysis were case-control study.<sup>37–40</sup> The demographic characteristics, study results and summary are described in tables 1 and 2, respectively. In the forest plot (figure 2), individuals with micronutrient deficiency were defined as an experimental group, while individuals with adequate status were classified as control group. Events were determined by number of individual infected with *H. pylori*.

### Overall meta-analysis

Overall, the four eligible studies represented data from four different countries including USA, Iran, Iraq, and Turkey. A total of 553 cases and 721 controls were recruited in the meta-analysis to find out association between micronutrients deficiency and *H. pylori* infection

among the pregnant women. As shown in figure 2, the overall meta-analysis in the fixed effect model suggested that the experimental (micronutrient deficient) group was not associated with *H. pylori* infection (OR=1.12, 95% CI 0.88 to 1.42, p=0.37) in comparison with control group (micronutrient sufficient). However, a moderate heterogeneity among the studies was observed (I<sup>2</sup>=70%, p<0.002).

### Subgroup meta-analysis

#### Association of vitamin B<sub>12</sub> with *H. pylori* infection

We compared the blood vitamin B<sub>12</sub> level in case control studies in which the patients were infected with *H. pylori*. A forest plot (figure 2) of 2 studies comprising 188 controls and 115 experimental participants (cases) were included for statistical analysis. There was no significant role of *H. pylori* infection in either group (OR=0.74, 95% CI 0.45 to 1.21, p=0.22). No evidence of statistical heterogeneity (I<sup>2</sup>=0%, p=0.75) was observed in the meta-analysis.

#### Association of folate with *H. pylori* infection

A subgroup analysis of folate was carried out. There was no significant effect of *H. pylori* infection on blood folate status in pregnant women (OR=1.04, 95% CI 0.73 to 1.58, p=0.73). From 3 studies, 333 controls and 270 cases were included for statistical pooling in the forest plot. Moderate heterogeneity was found in this subgroup with significant p value (I<sup>2</sup>=74%, p=0.05).

**Table 3** Subgroup metaanalysis of indicator of micronutrients

Subgroup	Included studies (n)	Participants (n)		OR (95% CI)	P value	Heterogeneity ( $\chi^2$ )
		Case	Control			
Vitamin B <sub>12</sub>	2	115	188	0.74 (0.45 to 1.21)	0.22	0.1
Folate	2	249	197	1.07 (0.73 to 1.58)	0.73	3.85
Ferritin	2	121	187	0.81 (0.51 to 1.31)	0.40	0.77
IDA	2	68	149	16.23 (4.19 to 62.93)	<0.001	0.41

### Association of iron status with *H. pylori* infection

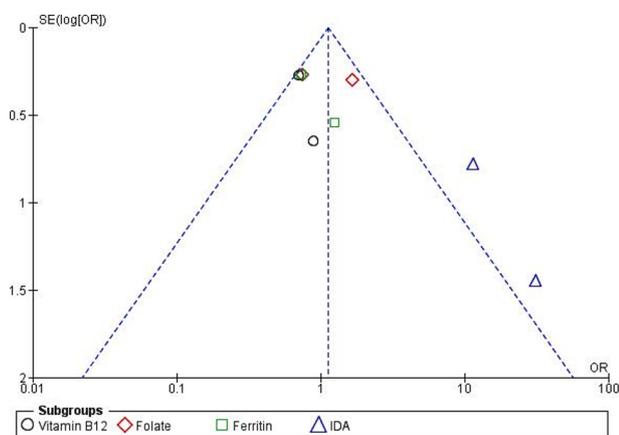
In usual conditions, iron status can be measured by assessing serum levels of ferritin.<sup>41</sup> Figure 2 showed that *H. pylori* infection was not associated to reduce the ferritin status (OR=0.81, 95% CI 0.51 to 1.31, p=0.4). From 3 studies, 171 cases and 316 control participants were pooled in the forest plot. However, no heterogeneity ( $I^2=0\%$ , p=0.38) was found in this subgroup.

Two studies reported a positive correlation between IDA status with *H. pylori* infection (OR=16.23, 95% CI 4.19 to 62.93, p<0.0001). Although the sample size was small (68 cases and 149 control), both studies described highly significant correlation of increasing the IDA condition when participants were infected with *H. pylori*. Minimal heterogeneity ( $I^2=0\%$ , p=0.52) was found in this subgroup.

From the forest plot (figure 2), it could be assumed that without IDA subgroup, the sign of the diamond might be directed to left side. When this subgroup was omitted, the overall result was not changed (OR=0.89, 95% CI 0.69 to 1.15, p=0.39), while the total heterogeneity decreased ( $I^2=0\%$ , p=0.25). The subgroup meta-analysis data were summarised in table 3.

### Publication bias

We created a funnel plot and Egger's test (figure 3) to measure the publication bias objectively for outcomes from four studies, although it is less powerful for the less than 10 studies.<sup>42</sup> No potential publication bias was found though some asymmetry was found in the funnel plot.



**Figure 3** Funnel plot with estimated 95% CIs for meta-analysis of the effect of *H. pylori* on micronutrient status. IDA, iron-deficiency anaemia.

### Quality assessment

The CASP checklist was used to evaluate the quality of the selected articles which include 10 questions to highlight the study rigour, biasness, research methods, risk factor, relevance and research integrity. We deduced a high percentage of interjudges' agreement (75.00%), while minimal were indecision (15.00%) and disagreement (10.00%). CASP scale score results showed that all of our enlisted studies had reasonable quality. The results of the quality assessment were summarised in table 4. Three studies (Golalipour *et al.*,<sup>37</sup> Felkner *et al.*<sup>38</sup> and Mubarak *et al.*<sup>36</sup>) had higher score (90%) and others three (Ali *et al.*,<sup>40</sup> Mulayim *et al.*<sup>39</sup> and Ugwuja *et al.*<sup>35</sup>) had 70%, 60% and 50% score that are considered as medium quality. The results of ROB assessment by using NOS was illustrated in supplementary materials. Two studies achieved low ROB (Ali *et al.*<sup>40</sup> and Mulayim *et al.*<sup>39</sup>) and other two studies achieved medium ROB (Golalipour *et al.*<sup>37</sup> and Felkner *et al.*<sup>38</sup>).

### DISCUSSION

The present meta-analysis shows that *H. pylori* infection in pregnancy has no major effect on the risk of micronutrients deficiency. Subgroup analysis also suggests that the infection is highly associated with increased risk of IDA during pregnancy but not with vitamin B<sub>12</sub>, folate, and ferritin. To date, this systematic review and meta-analysis have been most comprehensive and updated on the likely link between *H. pylori* and micronutrient deficiency in pregnant women.

In our meta-analysis, we included case-control and cross-sectional studies alone. Given the slew of potential confounding factors, RCTs might be more valuable in such case. However, due to divergence from the inclusion criteria, we could not include RCT, quasi-RCT, and other mixed-method studies. The stringent regulations for study inclusions which comprise the PICOS principle, study design, subject type, data availability, study quality, and adequate data to measure the effect size, restricted the scope for meta-analysis to include only four case control studies. In addition, two studies were discussed systematically in the result section. Although numerous literatures were screened, assessed, and selected methodologically, we found moderate heterogeneity in the overall meta-analysis ( $I^2=70\%$ ) with significant P value (p=0.002). This heterogeneity may be of clinical, methodological or statistical origin.<sup>43</sup> Characteristics of the

**Table 4** The Critical Appraisal Skills Programme (CASP) checklists results for assessing the methodological quality of the included studies

Questions	Ali <i>et al</i> 2017 <sup>40</sup>	Felkner <i>et al</i> 2007 <sup>38</sup>	Golalipour <i>et al</i> 2012 <sup>37</sup>	Mulayim <i>et al</i> 2008 <sup>39</sup>	Mubarak <i>et al</i> 2014 <sup>36</sup>	Ugwuja <i>et al</i> 2010 <sup>35</sup>
1 Did the study address a clearly focused issue?	Yes	Yes	Yes	Yes	Yes	Yes
2 Did the authors use an appropriate method to answer their question?	Yes	Yes	Yes	No	Yes	No
3 Were the cases recruited in an acceptable way?	Yes	Yes	Yes	Yes	Yes	Can't Tell
4 Were the controls selected in an acceptable way?	Yes	Yes	Yes	Yes	Yes	Yes
5 Was the exposure accurately measured to minimise bias?	Can't Tell	Yes	Yes	Yes	Yes	Can't Tell
6 Aside from the experimental intervention, were the groups treated equally?	Yes	Can't Tell	Can't Tell	Can't Tell	Can't Tell	Can't Tell
7 Have the authors taken account of the potential confounding factors in the design and/or in their analysis?	No	Yes	Yes	No	Yes	No
8 Do you believe the results?	Yes	Yes	Yes	Yes	Yes	Yes
09 Can the results be applied to the local population?	Can't Tell	Yes	Yes	No	Yes	Yes
10 Do the results of this study fit with other available evidence?	Yes	Yes	Yes	Yes	Yes	Yes
Individual study CASP score	7/10=70%	9/10=90%	9/10=90%	6/10=60%	9/10=90%	5/10=50%

Overall CASP score: Yes, 45/60=75.00%; Can't Tell, 9/60=15.00%; No, 6/60=10%.

study participants and severity of *H. pylori* infection may also cause high clinical heterogeneity since demographic traits cover a wide ranges data including age, BMI and gestational age. We assume that methodological heterogeneity does not affect overall heterogeneity. On the other hand, statistical heterogeneity may contribute to high heterogeneity since we have subgroups and some are inconsistent.

The association between *H. pylori* infection and vitamin B<sub>12</sub> status in pregnancy is a little investigated field of research. Earlier study reported the presence *H. pylori*-related gastritis in 57% of patients with macrocytic anaemia caused by B<sub>12</sub> deficiency.<sup>44</sup> Dietary vitamin B<sub>12</sub> is tightly bound to haptocorrin and optimal stomach pH

is essential to dissociate the vitamin from the protein enabling absorption.<sup>45</sup> Several studies revealed that *H. pylori* infection has dual effects on stomach pH; acute infection induces hypochlorhydria (higher pH) while chronic infections can exert either hyperchlorhydria or hypochlorhydria (lower pH), depending on the site of the infection.<sup>46 47</sup> However, our included studies are incapable to define individual patient's *H. pylori* infection statuses. Two studies corroborate our findings of no significant association between *H. pylori* infection and vitamin B<sub>12</sub> deficiency,<sup>48 49</sup> although these were among elderly populations. Another water-soluble B vitamin is folate, and its absorption is negatively influenced by higher pH triggered by *H. pylori* infection. A sample of either serum

or red blood cell (RBC) is commonly used in the quantification of folic acid. But assessment of serum folate is favoured by many clinicians compared with RBC folate.<sup>50</sup> Most importantly, RBC folate is superior to serum folate in pregnancy as it provides better folate status.<sup>51</sup> In our meta-analysis, half of the studies estimated folate from serum sample and remaining half estimated from RBC sample. This can probably explain our results which do not support the link between *H. pylori* positivity and reduced folate. Similar to our study, Rasool *et al*<sup>52</sup> found no impact of *H. pylori* on human serum vitamin B<sub>12</sub>, folate and homocysteine level.

Serum ferritin levels closely correlate with total body iron stores and measurement of the serum ferritin level is the most accurate test to diagnose IDA.<sup>53</sup> In this study, we found that *H. pylori* infection was not associated with ferritin concentrations. This lack of association may be confounded by the extent that *H. pylori* infection resides in the stomach. One study found that *H. pylori* positive patients with peptic ulcer disease had a negative correlation between *H. pylori* and serum ferritin.<sup>54</sup> In patients with normal gastrointestinal tract, no difference in ferritin levels were observed due to presence or absence of *H. pylori* infections.<sup>55</sup> Interestingly, we observed a strong positive correlation between *H. pylori* infection and IDA (OR=16.23, 95% CI 4.19 to 62.93, p<0.0001). Several cogent biological mechanisms exist through which *H. pylori* elicits iron depletion. First, *H. pylori* is responsible for haemorrhagic gastritis, gastric adenocarcinoma, and peptic ulcer disease, all of which increase iron loss.<sup>56</sup> Second, corporal gastritis attributable to *H. pylori* may curtail gastric acid secretion via gland atrophy, which would lead to decrease iron absorption.<sup>57</sup> Third, the CagA protein of *H. pylori* is involved in interstitial holotransferrin direct iron acquisition.<sup>58</sup> Iron uptake via the CagA protein further promotes *H. pylori* growth and intestinal colonisation.<sup>18</sup> Our results are supported by a meta-analysis of a case-control studies that confirmed an increased risk of IDA with *H. pylori* infection.<sup>59</sup>

There may have unspecified factors that affected our study findings. Misclassification of *H. pylori* infection is one of them, since *H. pylori* specific immunological parameters IgA and IgG are considered as diagnostic markers that differ in various studies. The half-life of the IgA and IgG immunoglobulins is greater than 21 days. Participants may have been free of *H. pylori* when specimens were collected, yet the antibody may have been in circulation due to prior infection.<sup>60</sup> The reverse was also possible, that is, subjects were infected during periconception but were no longer producing IgG or IgA antibodies at the time the blood specimens were drawn. In addition, cagA is a virulence factor for pathogenicity of *H. pylori* and it causes more severe gastric diseases with higher gastric pH.<sup>61–63</sup> The presence of cagA toxin in *H. pylori* could result in serious micronutrient deficiency. On the other hand, the absence of cagA toxin might be responsible for adequate micronutrient level although patients were infected with *H. pylori*. However,

our included studies did not detect this virulence factor. Further, in the studies where we extracted data, there were no indications of antibiotic or drug use. This additional information would have been valuable to include in our analysis because the uses of antibiotics can reduce nutritional absorbance.<sup>64 65</sup> Additional reasons for diminished micronutrient absorbance were other infections,<sup>66</sup> such as *Diphyllobothrium pacificum*<sup>67</sup> or *Giardia*,<sup>68</sup> which might cause iron and vitamin B<sub>12</sub> deficiency. Any other infection was not clearly described in the studies we meta-analysed.

## CONCLUSION

*H. pylori* infection and micronutrient deficiencies are both endemic and widespread in economically disadvantaged populations especially in pregnant women. This meta-analysis shows that *H. pylori* infection is associated with increased risk of IDA but not with vitamin B<sub>12</sub>, folate, and ferritin during pregnancy. Therefore, it is important to screen *H. pylori* infection, especially for effective treatment of IDA in pregnant women. Our result is inconclusive due to limitation of the study number, insufficient data, weakness of the studies and current epidemiological evidences. Additional well-designed longitudinal studies are warranted to clarify the role of *H. pylori* infection that may elicit micronutrient deficiency during gestation. We recommend several important factors that must be considered for future study design; these factors are the incidence of *H. pylori* infection, the magnitude of infected area, duration of infection, dietary information, drug uses and other subclinical infections prior to and during the pregnancy.

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