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ORIGINAL ARTICLE

Association between *Helicobacter pylori* Infection and Graves' Disease: A Meta-Analysis

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ABSTRACT

Background: *Helicobacter pylori (H. pylori) infection is proposed to be related with autoimmune diseases, such as Graves' disease. This study aimed to assess the association between H. pylori infection and Graves' disease.*

Method: *A systematic literature review was conducted using Pubmed and Cochrane library. The quality of enrolled studies was assessed by the Critical Appraisal Skills Program Oxford. A fixed-effect model approach was used if there was no heterogeneity; otherwise, a random-effect model was used. Heterogeneity was assessed using I^2. Publication bias was assessed by funnel plot. All data were analyzed using REVIEW MANAGER 5.3.*

Results: *Six studies from Europe and Asia involving 983 patients were included. Overall H. pylori infection was significantly associated with Graves' disease (OR = 2.7; 95% CI: 1.47-4.99; $p < 0.001$). In subgroup analysis of 3 studies using non-serological diagnostic method, the prevalence rate of H. pylori infection was higher in Graves' disease group (78.26% vs. 42.42%) with significant relationship (OR = 4.93; 95% CI: 3.16-7.69; $p < 0.00001$; $I^2 = 0\%$). The Cytotoxin associated gene A (CagA) antibody prevalence was significantly higher in Graves' disease group (46.57% vs. 20.29%; OR = 4.41; 95% CI: 2.65-7.33; $p < 0.00001$; $I^2 = 56\%$). No publication bias was observed.*

Conclusion: *Our study showed association between H. pylori infection and Graves' disease. It might suggest the need of H. pylori examination in Graves' disease patients and the impact of H. pylori eradication in the treatment of Graves' disease.*

Keywords: *Helicobacter pylori, Graves' disease, meta-analysis*

ABSTRAK

Latar belakang: *Beberapa penelitian melaporkan infeksi Helicobacter pylori (H. pylori) berkaitan dengan penyakit otoimun, antara lain Graves' disease. Penelitian ini bertujuan untuk mengevaluasi hubungan antara infeksi H. pylori dengan Graves' disease.*

Metode: *Telaah literatur dilakukan dengan menggunakan Pubmed dan Cochrane library. Kualitas studi dinilai dengan Critical Appraisal Skills Program Oxford. Pendekatan fixed-effect model digunakan jika tidak terdapat heterogenitas dan random-effect model digunakan jika terdapat heterogenitas. Heterogenitas dievaluasi dengan I^2. Bias publikasi dikaji dengan funnel plot. Semua data dianalisis dengan program REVIEW MANAGER 5.3.*

Hasil: *Enam studi dari Eropa dan Asia meliputi 983 pasien masuk ke dalam kriteria inklusi. Secara keseluruhan, infeksi H. pylori secara signifikan berhubungan dengan Graves' disease (OR = 2.7; 95% CI:*

1.47-4.99; p < 0.001). Pada analisis subgrup dari 3 studi yang menggunakan metoda diagnostik non-serologik, didapatkan prevalensi infeksi H. pylori yang lebih tinggi secara bermakna pada grup Graves' disease (78.26% vs. 42.42%; OR = 4.93; 95% CI: 3.16-7.69; p < 0.00001; I^2 = 0%). Prevalensi antibodi CagA secara signifikan lebih tinggi pada grup Graves' disease (46.57% vs. 20.29%; OR = 4.41; 95% CI: 2.65-7.33; p < 0.00001; I^2 = 56%). Tidak ditemukan adanya bias publikasi.

***Simpulan:** Penelitian ini menunjukkan hubungan antara infeksi H. pylori dengan Graves' disease. Hal ini dapat mendasari perlunya pemeriksaan H. pylori pada pasien Graves' disease dan dampak eradikasi H. pylori dalam terapi Graves' disease.*

***Kata kunci:** Helicobacter pylori, Graves' disease, meta-analisis*

INTRODUCTION

Graves' disease, having thyrotoxicosis as clinical hallmark, is characterized by formation of autoantibody thyroid stimulating immunoglobulins (TSI) to the thyroid stimulating hormone receptor (TSH-R). Other antibodies, thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb), might also present in Graves' disease. The etiology of Graves' disease is considered as a complex combination of genetic and environment factors.[1,2]

The discovery of *Helicobacter pylori* (*H. pylori*) by Marshall and Warren in 1982 has contributed significantly in understanding pathogenesis of diseases. Globally, the prevalence of *H. pylori* infection is more than 50%. The impact of this microaerophilic, gram negative curved bacillus is not just limited at the gastrointestinal; moreover, it has been proposed to have extra-gastrointestinal manifestations, such as coronary heart disease, diabetes mellitus, autoimmune diseases.[3-7] The presence of seropositivity to the cytotoxin-associated gene A (Cag-A) is commonly used to identify the virulence of *H. pylori*.[6,7]

The interaction of *H. pylori* as infectious environmental exposure and genetic susceptibility resulting in autoimmune disorder such as Graves' disease has become an appealing issue.[1,3,4,8] The present study was performed to evaluate the association between *H. pylori* infection and Graves' disease through review of existing studies.

METHOD

We conducted this study according to the meta-analysis PRISMA guideline (see PRISMA checklist).[9] We performed computerized literature search of Cochrane and PubMed database up to July 2015. For the search, we limited to humans and used the following keywords: "*Helicobacter pylori*" or "*H. pylori*", and "Graves' disease". We also searched manually related references cited by the original published studies and relevant review articles. Article selection and assessment were done by reviewers. We contacted the authors via email to obtain the required information when relevant information was not available in the published article.

Studies were included for analysis based on the following inclusion criteria: (i) observational study having control group; (ii) the outcome was Graves' disease; (iii) Graves' disease was diagnosed by the presence of hyperthyroidism (suppressed TSH, elevated FT3, elevated FT4), diffuse goiter with positive antibody titers (TSI, TPOAb, TgAb), and, in some cases, opthalmopathy; (iv) the exposure was *H. pylori* infection; (v) the diagnosis of *H. pylori* infection was based on serological tests (antigen-specific enzyme-linked immunosorbent assay (ELISA) and Western blotting) or non-serological tests (rapid urease test, stool antigen test (SAT), ^{13}C-urea breath test (UBT)); (vi) studies had extractable data and sufficient information on the association between *H. pylori* infection and Graves' disease.

We also recorded the CagA serology examination result if it was done in the study. Since all parameter observed were objectively measured and having a written record, neither recall bias nor observer bias were occur in each study. We assessed the quality of each study by using the criteria from the critical appraisal skills program (CASP) Oxford United Kingdom consisting of 11 systematic questions for appraising case control study. The quality levels then were graded as good, fair, and poor.[10] Only studies with good quality were included in our final analysis review. Discrepancies and disagreements were resolved by consensus.

Study characteristics were taken as follows: first author; year of publication; study design; country; *H. pylori* test method; subjects characteristics (case subjects, age in mean or median, sex, and matched

between two groups); CagA test availability; number of subjects with positive *H. pylori* test in each outcome group; number of subjects with positive CagA test in each outcome group.

We calculated the odds ratios (OR) with 95% confidence interval (CI) for *H. pylori* positivity. The Mantel-Haenszel method was used to weight the studies included. A fixed-effect model approach was use if there was no heterogeneity; otherwise, a random-effect model was used. Heterogeneity was assessed using I^2. Negative value of I^2 was put equal to 0. I^2 values ranged from 0% (no observed heterogeneity) to 100%, and interpreted according to Cochrane Consumers and Communication Review Group.[11] For sub-analysis, we calculated the OR of CagA seropositivity. Moreover, to evaluate the possible bias due to difference in diagnostic method, we did sub-analysis of the OR of *H. pylori* positivity by serological diagnostic method and the OR of *H. pylori* positivity by non-serological diagnostic method (study using both serological and non-serological diagnostic method will be included here). Publication bias was assessed by funnel plot. All statistical analysis was performed using Review Manager 5.3.

RESULTS

Our literature search identified 16 studies, all published in English. We excluded studies which were not cohort, or case control studies (n = 5), had no available information on the association between *H. pylori* infection and Graves' disease (n = 2), had no control group (n = 1). After final-text screening, we decided to exclude 1 study due to different baseline profile of *H. pylori* infection and 1 study due to quality issue. Finally, a total of 6 studies involving 983 patients met our criteria. The flowchart showed the process of studies selection (Figure 1).

The studies were published between 1998 and 2013, and the characteristics of which are summarized in Table. Four studies were performed in Europe, and 2 studies were performed in Asia.[7,8,12-15] Four studies had Graves' disease subjects as a subgroup of the case group (subjects with autoimmune thyroid disease) and 2 other studies had Graves' disease subjects as the whole subjects of case group.[7,8,12,15] All studies had greater number of female than male subjects in both Graves' disease and control groups. None of the studies performed endoscopy due to young age subjects and had no alarm symptoms. Three studies used serology method, 2 studies used SAT method, and 1 study used combination of serology and UBT method[12] to assess the infection status of *H. pylori*. Four studies examined CagA antibody to identify infection with *H. pylori* strain possessing CagA.[7,8,13,14,15]

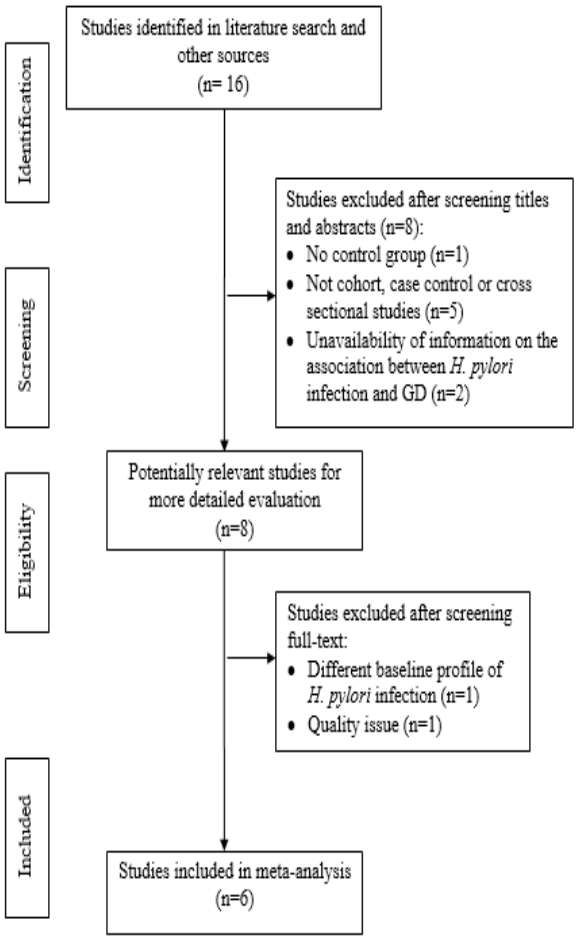

Figure 1. Flow diagram of literature selection

Table 1. Characteristic of studies included in meta-analysis

Author	Year of publication	Study design	Country	HP test method	Case subjects	Age (mean or median)
De Luis[12]	1998	CC	Spain	UBT & ELISA	ATD (Graves' disease as subgroup) patients	33.9 ± 1.2
Larizza[14]	2006	CC	Italy	ELISA	ATD (Graves' disease as subgroup) patients	11.7
Bassi[7]	2010	CC	Italy	SAT	Graves' disease patients	42.8 ± 8.8
Soveid[15]	2012	CC	Iran	ELISA	ATD (Graves' disease as subgroup) patients	36.3 ± 7.6
Bassi[13]	2012	CC	Italy	SAT	ATD (Graves' disease as subgroup) patients	48.8 ± 3.9
Wang[8]	2013	CC	China	ELISA	ATD (Graves' disease as subgroup) patients	28.0 ± 5.4

CC: Case control; NS: not specified; ATD: Autoimmune thyroid disease

Table 2. Characteristic of studies included in meta-analysis

Author	Sex (F/M) Graves' disease	Sex (F/M) Control	Matched between case & control					CagA test
			Age	Sex	S	SE	Area	
De Luis[12]	13/7	21/10	Yes	Yes	Yes	NA	Yes	NA
Larizza[14]	23/2	55/15	Yes	Yes	NA	NA	Yes	NA
Bassi[7]	98/14	87/13	Yes	Yes	Yes	Yes	Yes	A
Soveid[15]	38/5	89/23	Yes	Yes	NA	Yes	Yes	A
Bassi[13]	48/4	90/10	Yes	Yes	Yes	Yes	Yes	A
Wang[8]	122/94	59/43	Yes	Yes	Yes	NA	Yes	A

HP: *Helicobacter pylori*; F: female; M: male; A: available; NA: not available; S: smoking; SE: socioeconomic; ELISA: enzyme-linked immunosorbent assay; UBT: 13C-urea breath test; SAT: stool antigen test; CagA: cytotoxin associated gene A

Table 3. Characteristic of studies included in meta-analysis

Author	HP+ Graves' disease	HP+ control	CagA+ Graves' disease	CagA+ control
De Luis[12]	13	13	NA	NA
Larizza[14]	8	9	NA	NA
Bassi[7]	88	42	66	20
Soveid[15]	32	87	20	28
Bassi[13]	43	43	36	21
Wang[8]	143	53	75	15

HP: *Helicobacter pylori*; CagA: cytotoxin associated gene A

Prevalance rate of *H. pylori* positivity showed statistically significant different between Graves' disease and control group in 4 studies.[7,8,13,14] One study showed higher prevalence of *H. pylori* positivity in Graves' disease group although it was not statistically significant.[12] One study, in contrary, showed higher prevalence of *H. pylori* positivity in control group but not statistically significant.[15] The overall prevalence rate of *H. pylori* positivity was 69.87% (327 of 468) in Graves' disease group and 47.96% (247 of 515) in control group. Positivity of *H. pylori* infection was significantly associated with Graves' disease (pooled OR = 2.7; 95% CI: 1.47-4.99; test for overall effect Z = 3.18; p < 0.001). However, there was a substantial heterogeneity (I^2 = 74%).

We performed subgroup analysis of study using different method of diagnostic test. In the 3 studies using serological diagnostic method, the prevalence rate of *H. pylori* infection was higher in Graves' disease than in control group (64.43% [183 of 284] vs. 52.46% [149 of 284]). However, the association was not significant (pooled OR = 1.61 [95% CI: 0.85-3.06, test for overall effect Z = 1.47, p 0.14]) with moderate heterogeneity (I^2 = 52%) (Figure 2).

In the 3 studies using non-serological diagnostic method, the prevalence rate of *H. pylori* infection was also higher in Graves' disease group than in control group (78.26% [144 of 184] vs. 42.42% [98 of 231]). Moreover, the relationship was statistically significant (pooled OR = 4.93 [95% CI: 3.16-7.69, test for overall effect Z = 7.03, p < 0.00001]). There was no heterogeneity existed (I^2 = 0%) (Figure 3).

The CagA antibody prevalence in the pooled 4 studies was higher in Graves' disease group (46.57% [197 of 423]) than in control group (20.29% [84 of 414]) with significant OR = 4.41 (95% CI: 2.65-7.33), test for overall effect Z = 5.70, p < 0.00001). The heterogenity was moderate (I^2 = 56%) (Figure 4).

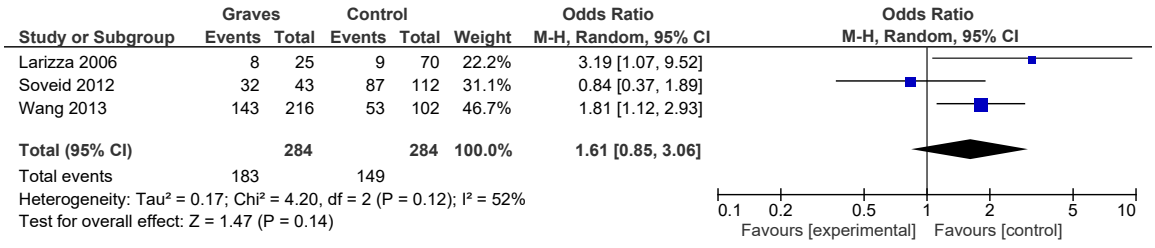

Figure 2. Association between *H. pylori* infection and Graves' disease in studies using serological diagnostic method

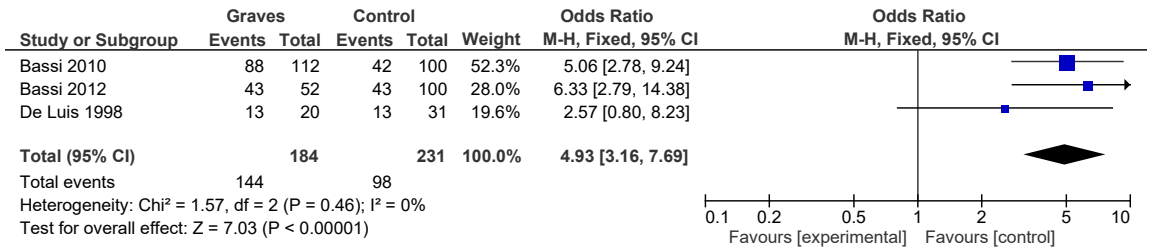

Figure 3. Association between *H. pylori* infection and Graves' disease in studies using non-serological diagnostic method

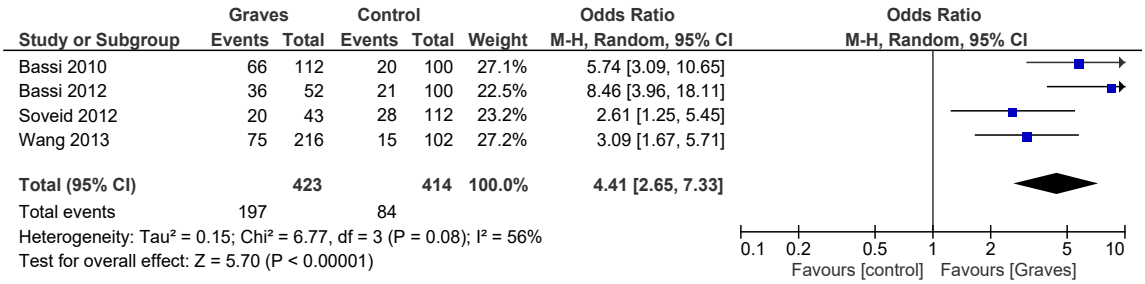

Figure 4. Association between CagA seropositivity and Graves' disease

For the overall 6 studies, no evidence of publication bias was observed in the funnel plot (Figure 5).

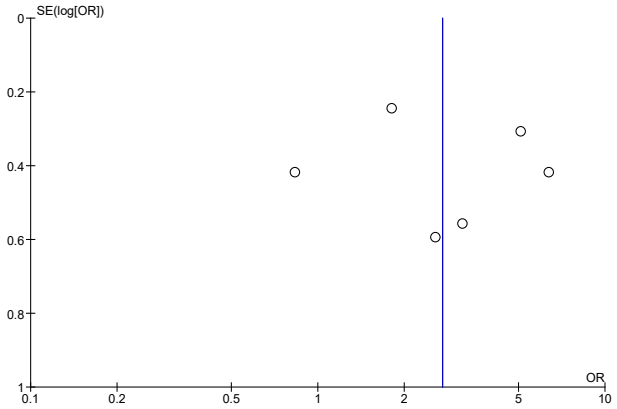

Figure 5. Funnel plot analysis of *H. pylori* infection and Graves' disease

DISCUSSION

Based on meta-analysis of 6 studies, there was a significant association between *H. pylori* positivity and Graves' disease. Graves' disease, a member of autoimmune thyroid disease, arises from the complex interaction of genetic susceptibility and environmental factor. Infectious agent is one of the environmental factors receiving particular attention. Among infectious agents, *H. pylori* infection has been proposed to trigger the Graves' disease by several mechanisms. Earlier studies showed that the lipopolysaccharide (LPS) of *H. pylori* has a similar structure with human cell surface glycoconjugates Lewis X, Lewis Y. The "antigenic mimicry" expression of Lewis determinant in LPS is associated with CagA status of the *H. pylori* strain.[16-18] This "antigenic mimicry" might induce autoimmune response which influences the thyroid gland. Interestingly, *H. pylori* might play role through modulation of dendritic cells, T helper cells, resulting in expression of proinflammatory cytokines such as interferon (IFN)-gamma, interleukin (IL)-10, IL-17.[19-21]

We performed subgroup analysis by separating the diagnostic methods into serologic and non-serologic. As we know, serological examination of antibody to *H. pylori* cannot differentiate either past or current infection. Moreover, the background of *H. pylori* prevalence influences the positive predictive value (PPV) of this method, as discussed in the study by Soveid in Iran.[15-22] We found no significant association between *H. pylori* seropositivity and Graves' disease in this subgroup, as shown by the pooled OR of 3 studies using serology method, and moderate heterogeneity. In contrary, the association was statistically significant in the non-serology method subgroup with 0% of heterogeneity. This finding may underline the importance of ensuring the infection status (current infection) since the antibody itself can persist even after eradication therapy. Therefore, either UBT or SAT method is considered as the preferred non-invasive diagnostic method.

For the subgroup analysis of CagA seropositivity, overall studies showed that infection by the more virulent strain (CagA seropositive) group was significantly correlated with Graves' disease. The possession of CagA plays role in the development of Graves' disease through "antigenic mimicry" and cytokines expression.[17,19] Different diagnostic kit used in each study may contribute to the heterogeneity.

In this study, we had heterogeneous race of subjects, consisting of Caucasian (4 studies), Asian Chinese (1 study), and Asian Iranian (1 study). Genetic factors, which may be clustered more in people depending on their race might play role in the development of Graves' disease. However, there were just 2 studies specifically assessed the genetic factor (HLA alleles), one in Caucasian and one in Asian Chinese. Unfortunately, the result of both studies was not supporting each other.[8,14] Additional studies need to be done to confirm the specific alleles preventing or increasing risk developing of Graves' disease in correlation with *H. pylori* infection.

To our knowledge, this is the first meta-analysis study to investigate the association between *H. pylori* positivity and Graves' disease. This association might give an idea of the need *H. pylori* examination in

Graves' disease patients and further investigation whether the treatment of *H. pylori* will positively influence the treatment of Graves' disease. However, this study has some potential limitations. First, since all studies collected the data of exposure and outcome in the same time, temporal relationship could not be clearly defined. Second, there were just six studies met the criteria and were included the meta-analysis.

CONCLUSION

Our study showed association between *H. pylori* infection and Graves' disease. We realize that more longitudinal studies with uniform diagnostic method, larger sample sizes, and multiethnic are needed for better understanding of *H. pylori* and Graves' disease relationship.

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