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CagA toxin and risk of *Helicobacter pylori*-infected gastric phenotype: A meta-analysis of observational studies

Cho Naing[1☯]*, **Htar Htar Aung**[2☯]*, **Saint Nway Aye**[2], **Yong Poovorawan**[3], **Maxine A. Whittaker**[1]

1 College of Public Health, Medical and Veterinary Sciences, James Cook University, Queensland, Australia,
2 School of Medicine, IMU University, Kuala Lumpur, Malaysia, **3** Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

☯ These authors contributed equally to this work.
* cho3699@gmail.com (CN); htar.ng@gmail.com (HHA)

Abstract

Background

Helicobacter pylori (*H. pylori*) is frequently associated with non-cardia type gastric cancer, and it is designated as a group I carcinogen. This study aimed to systematically review and meta-analyze the evidence on the prevalence of CagA status in people with gastric disorders in the Indo-Pacific region, and to examine the association of CagA positive in the risk of gastric disorders. This study focused on the Indo-Pacific region owing to the high disability adjusted life-years related to these disorders, the accessibility of efficient treatments for this common bacterial infection, and the varying standard of care for these disorders, particularly among the elderly population in the region.

Methods

Relevant studies were identified in the health-related electronic databases including PubMed, Ovid, Medline, Ovid Embase, Index Medicus, and Google Scholar that were published in English between 1 January 2000, and 18 November 2023. For pooled prevalence, meta-analysis of proportional studies was done, after Freeman-Tukey double arcsine transformation of data. A random-effect model was used to compute the pooled odds ratio (OR) and 95% confidence interval (CI) to investigate the relationship between CagA positivity and gastric disorders.

Results

Twenty-four studies from eight Indo-Pacific countries (Bhutan, India, Indonesia, Malaysia, Myanmar, Singapore, Thailand, Vietnam) were included. Overall pooled prevalence of CagA positivity in *H. pylori*-infected gastric disorders was 83% (95%CI = 73–91%). Following stratification, the pooled prevalence of CagA positivity was 78% (95%CI = 67–90%) in *H. pylori*-infected gastritis, 86% (95%CI = 73–96%) in peptic ulcer disease, and 83% (95%CI = 51–100%) in gastric cancer. Geographic locations encountered variations in CagA

prevalence. There was a greater risk of developing gastric cancer in those with CagA positivity compared with gastritis (OR = 2.53,95%CI = 1.15–5.55).

Conclusion

Findings suggest that the distribution of CagA in *H. pylori*-infected gastric disorders varies among different type of gastric disorders in the study countries, and CagA may play a role in the development of gastric cancer. It is important to provide a high standard of care for the management of gastric diseases, particularly in a region where the prevalence of these disorders is high. Better strategies for effective treatment for high-risk groups are required for health programs to revisit this often-neglected infectious disease.

Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that colonizes the stomach and is attributed to causing infections in humans [1, 2]. As *H. pylori* is frequently associated with non-cardia type of gastric cancer, and it is designated as a group I carcinogen [3]. Studies reported that only 20% of the colonizing bacteria at any time directly interact with the stomach epithelial cells [4], which are insufficient to cause gastric disorder [5]. This reflects that, in addition to its urease activity, *H. pylori* possesses additional virulence factors that are required to enable it to colonize and survive in the stomach's acid environment [4, 5]. The underlying mechanism of infectious diseases is greatly impacted by strain variations, which could account for the different outcomes of *H. pylori* infection. Studies have documented that *H. pylori* bacterial strains exhibit significant genetic diversity. Such diversity could result in an array of clinical outcomes, including gastritis, peptic ulcer disease, gastric adenocarcinoma (gastric cancer), and lymphoma. These outcomes depend on a variety of factors, particularly the virulence determinants expressed by distinct strains of *H. pylori* [6, 7]. Studies have reported that virulent determinants such as cytotoxin-associated gene A (CagA), lipopolysaccharide, vacuolating cytotoxin, peptidoglycan, and gamma-glutamyl transpeptidase are associated with gastric disease [8]. Among these, CagA is expressed by the terminal *cag*A gene of the type IV secretion system (T4SS) and discovered on the cag pathogenicity island (cagPAI) [9]. T4SS specifically forms a needle-like pilus device that is used to inject virulence factor such as the CagA effector protein into host target cells [10–13]. *H. pylori* strains with CagA-positive are more virulent than CagA-negative bacteria because they are linked to severe gastric inflammation [14].

With regards to gastric phenotype, there is an enigma coined as the "Asian Enigma" [15] or "Asian Paradoxical" [16] that affects many Asian countries [17, 18]. It draws attention to the need to study whether CagA prevalence is related to this paradoxical pattern. Several studies assessed CagA frequency in gastric disorders, which showed different findings. Since *H. pylori*-infected patients showed variations in the clinical manifestations such as gastritis, peptic ulcers, and gastric cancer, reporting the CagA prevalence on a range of gastric disorders is of immense value. Additionally, a recent review of the Global Burden of Disease data reported that Indo-Pacific super-, region has the highest disability-adjusted life years (DALY) burden for more digestive disease types, including oesphageal disorders and ulcers (17%) globally. These disorders can cause considerable distress and disability for those who suffer from them, as well as a large burden on the health care system and its financing [19]. Moreover, some socioeconomic groups within the countries of this region have been shown to have poorer 'Quality of Care Index' (e.g. access to effective treatment) for peptic ulcer disease [20]. It is

imperative to emphasize the significance of a common and treatable bacterial infectious disease [21] as a cause of peptic ulcer disease in these settings, as countries struggle with the rising expenses of providing universal health care for their citizens and an aging population that is frequently underserved for gastric disorders, including peptic ulcers [20].

Taking all these into consideration, the objectives of this study were to systematically review and meta-analyze the evidence on the prevalence of CagA status in people with gastric disorders in the Indo-Pacific region, and to examine the association of CagA positive in the risk of gastric disorders.

Materials and methods

This study followed the PRISMA-2020 checklist [22] [S1 Checklist]. The protocol of this study is available from the corresponding author upon reasonable request. The consent from the participants was not necessary as the current study used solely published data.

Search strategy

Two investigators (CN, HHA) looked at the relevant studies in health-related electronic databases including PubMed, Ovid, Medline, Ovid Embase, Index Medicus, and Google Scholar. The search terms used were (*Helicobacter pylori*, *H. pylori*, virulence factor, CagA, gastric cancer, gastritis, and peptic ulcer) with appropriate Boolean operators (AND/OR). The search was confined to publications in English between January 1 2000 and November 18 2023. Furthermore, hand-searching took place following the same inclusion criteria set for this study. Search Strategy in PubMed is provided in S1 Table.

Selection criteria

Studies were considered if they met the following criteria.

1. Human studies of observational design that assessed adults with *H. pylori*-infected gastric disorder.

2. Conducted in low- and middle-income Indo-Pacific countries.

3. Assessed the CagA status, irrespective of the detection method.

4. Provide sufficient data to compute the prevalence of CagA, and/or association of CagA with any or all three gastric disorders (i.e., gastritis, peptic ulcer disease, gastric cancer)

5. Examined the presence of genotypes with the use of PCR.

6. Included a minimum of ten samples.

In this study, the participants with duodenal and/or gastric ulcerations were categorized as peptic ulcer disease as stated in the primary studies. Endoscopic gastritis was regarded as an equivalent to 'non-ulcer disorder' with neither a history of ulcer disease nor endoscopic evidence of ulcer disease [23].

Exclusion criteria

Studies that did not satisfy the criteria for inclusion were not considered. In addition, reviews, letters, editorials, efficacy trials, and modelling studies were omitted.

Data collection

One investigator (CN) screened the title and abstracts, which were deemed eligible. Two investigators (HHA, CN) independently selected studies from those saved from the databases. Full-text studies that were deemed appropriate were further reviewed. Any discrepancy between the two investigators was settled by agreement. Using a self-created data extraction sheet, one investigator (CN) gathered data from the eligible studies. Another investigator (HHA) cross-checked this. Data collection included first author, year of publication, study country, study design, prevalence (numerator and denominator), participant characteristics (age, sex), types of gastric disorders (gastritis, peptic ulcer, gastric cancer), and methods of DNA sequencing.

Assessment of the quality of studies

The methodological quality of the studies involved in this study was rated using the Newcastle-Ottawa scale (NOS) checklist [24]. This tool has a star rating as a ranking scheme for observational studies, where nine stars are the maximum obtainable. The higher star ranking obtained, the better quality of the study.

Data analysis

At the individual-study level, the prevalence (along with variance) of CagA in a certain gastric phenotype was estimated by dividing the number of CagA-positive cases by the total number of cases examined. For pooling of studies, a proportional meta-analysis was carried out as described by Barker and associates [25], after variance normalization with the Freeman-Tukey double arcsine transformation [26]. For the association of CagA and the development of gastric disorder, a pooled odds ratio (OR) with 95% CI was estimated.

Concerning the variations among studies, the DerSimonian and Laird random effect model was employed for computing summary estimates [27]. An I^2 value of >75% denotes substantial variation [28]. Publication bias was not assessed for proportional meta-analysis owing to a methodological shortcoming in Egger's tests and funnel plots [25]. As there was a sufficient number of studies, Egger's test was used to gauge publication bias for the risk comparisons between gastritis and peptic ulcers. A simple test of asymmetry of the funnel plot (plot of effect estimates against sample size) was visualized [29].

Data analyses were done with the "*metaprop_one*" command for proportional meta-analysis, "*metan*" command for binary associations, and "*meta*" for publication bias command in STATA (version 15) (TXT, USA).

Results

Fig 1 illustrates the PRISMA flow diagram indicating the study selection process in the present meta-analysis. A total of 24 studies (26 datasets) were identified across eight Indo-Pacific countries such as Bhutan, India, Indonesia, Malaysia, Myanmar, Thailand, Singapore, and Vietnam [30–53]. Publication years spanned from 2005 [46] to 2023 [42]. A list of excluded studies along with their justifications is provided in S2 Table.

Table 1 presents the main characteristics of the 24 studies included in the current meta-analysis. Most of the studies were conducted in Thailand (6 studies) and Malaysia (6 studies). These individual studies examined a variety of gastric disorders, the most common being gastritis (23 studies), followed by peptic ulcer disease (17 studies), and gastric cancer (11 studies). The Trang study reported data from three countries, Bhutan, Myanmar and Vietnam [49]. Fifteen studies reported data on comparison of CagA positive and negatives in gastric disorders [31, 33–37, 41–46, 49, 52, 53]. The methodological quality of the studies identified was a
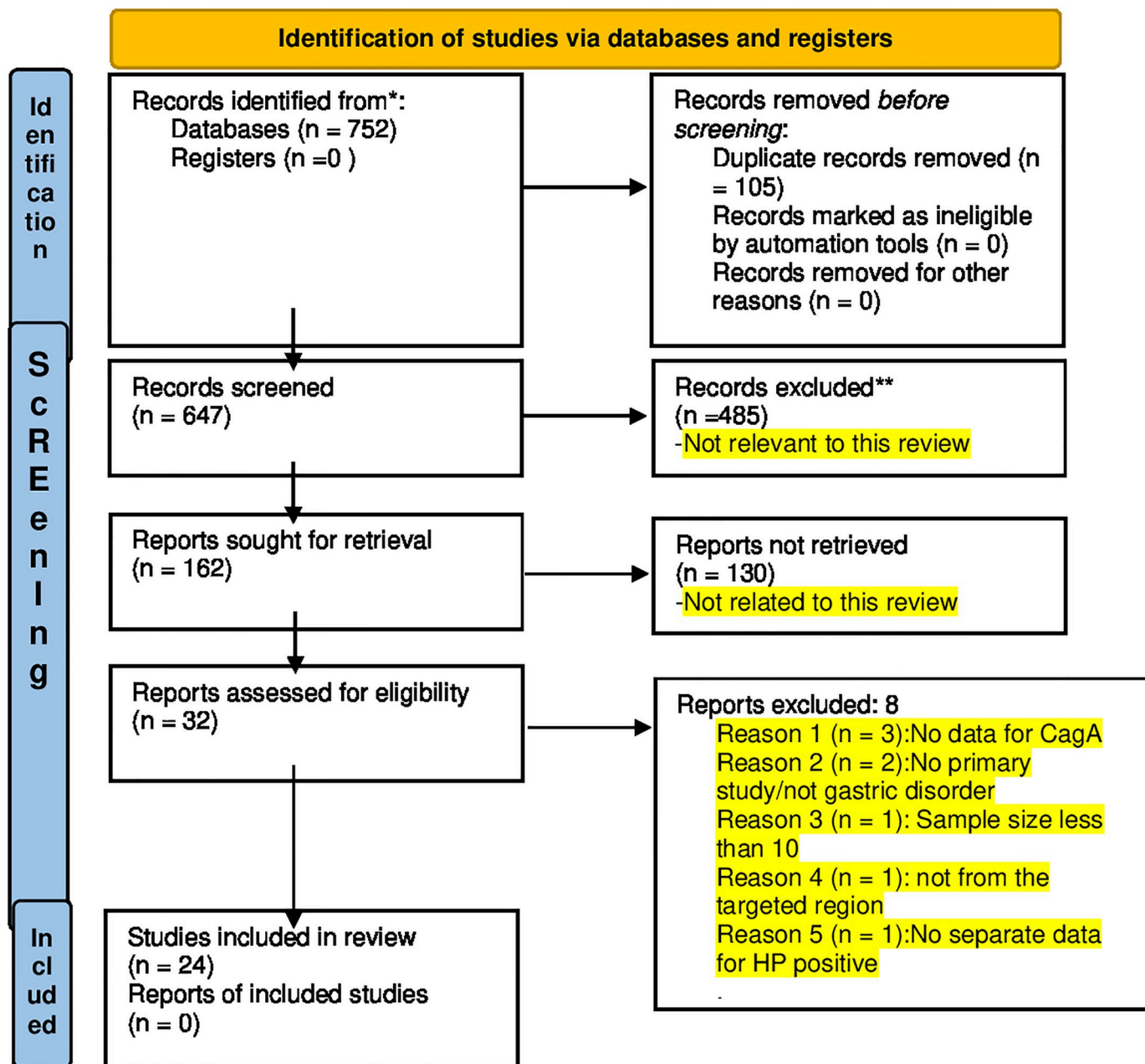
Identification of studies via databases and registers

Records identified from*:
Databases (n = 752)
Registers (n =0)

Records removed *before screening*:
Duplicate records removed (n = 105)
Records marked as ineligible by automation tools (n = 0)
Records removed for other reasons (n = 0)

Records screened (n = 647)

Records excluded** (n =485)
-Not relevant to this review

Reports sought for retrieval (n = 162)

Reports not retrieved (n = 130)
-Not related to this review

Reports assessed for eligibility (n = 32)

Reports excluded: 8
Reason 1 (n = 3):No data for CagA
Reason 2 (n = 2):No primary study/not gastric disorder
Reason 3 (n = 1): Sample size less than 10
Reason 4 (n = 1): not from the targeted region
Reason 5 (n = 1):No separate data for HP positive

Studies included in review (n = 24)
Reports of included studies (n = 0)

Fig 1. PRISMA flowchart indicating the study selection process.

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median of 6 stars (range 5–8 stars), reflecting most of the studies were of moderate quality (S3 Table).

Prevalence of CagA in *H. pylori*-infected gastric disorders

The CagA prevalence in *H. pylori*-infected gastric disorders was assessed in 24 studies, irrespective of the specific type (Fig 2). The overall pooled prevalence of CagA in *H. pylori*-infected gastric disorders was 83% (95%CI = 73–91%). This indicated that the great majority of

Table 1. Characteristics of the studies identified (N = 24).

No.	Study [ref #]	Yr	Country	Design	Mean age, yr	Male n(%)	Total	CagA+ve/tested samples			Remark
								GT	PUD	GC	
1	Myint [40]	2018	Myanmar	CS	40.1±11.	28 (57)	69	61/69	NA	NA	3DU+1 GC
2	Ansari [32]	2017	Myanmar	CS	40.7± 11.5	30 (24)	72	67/72	NA	NA	GT 89.3%;a subset data
3	Jeyamani [36]	2018	India	CS	NA	NA	61	16/35	12/14	0/0	
4	Linpisarn [37]	2007	Thailand	CS	NA	NA	135	48/58	68/73	3/4	
5	Boonyanugomol [33]	2020	Thailand	CS	NA	NA	80	38/50	17/20	8/10	
6	Nguyen [42]	2023	Vietnam	CS	9.1± 2.4	NA	269	0	185	NA	
7	Nguyen [41]	2010	Vietnam	CS	NA	NA	100	71	24	NA	
8	Pandya [43]	2017	India	CS	R:10–90	NA	43	9/34	6/9	NA	
9	Chomvarin [34]	2008	Thailand	CS	49.5	55 (49)	112	60/62	34/34	16/16	
10	Chomvarin [35]	2012	Thailand	CS	50 (R: 15–81	77 (52)	147	65/68	52/57	17/18	Mixed/ unclassified not included.
11	Yamada [52]	2013	Thailand	CS	56 ± 11.3[1]	93(42)	220	34/134	NA	7/86	Benign = GT; 1 = GC group
12	Zheng [53]	2000	Singapore	CS	NA	NA	108	37/41	58/67	NA	
13	Trang [49]	2015	Vietnam	CS	44.1 ± 12.7	47 (46)	102	72/76	25/26	NA	
			Myanmar	CS	40.1 ± 11.7	28 (41)	66	59/66	NA	NA	
			Bhutan	CS	36.8 ± 13.4	94 (47)	200	161/161	38/38	NA	
14	Uchida [51]	2009	Thailand	CS	45	47 (45)	103	98	NA	NA	25 = PUD
15	Alfizah [30]	2012	Malaysia	CS	56.05 ± 12.95	58 (53)	110	81/81	28/28	NA	
16	Mohamed [39]	2009	Malaysia	CS	NA	NA	93	44/93	26/93	NA	Based on the total tested.
17	Miftahussurur [38]	2015	Indonesia	CS	47.8 ± 14.6	NA	44	43	0	0	8 Other types are included.
18	Tiwari [48]	2011	India	CS	NA	NA	32	3	15	14	
19	Ali [31]	2005	India	CS	48.4	100 (57)	174	4/56	53/104	12/14	
20	Tan [46]	2005	Malaysia	CS	55.53 ± 12.52	72(57)	127	59/75	25/28	0/2	
21	Tan [47]	2006	Malaysia	CS	53.38 ± 12.09	36 (49)	73	58/73	NA	NA	
22	Truong [50]	2009	Vietnam	CS	NA	NA	22	NA	11/11	11/11	
23	Schmidt [44]	2009	Malaysia	CS	NA	NA	127	105/105	NA	21/21	
24	Schmidt [45]	2010	Malaysia	CS	NA	87 (55)	159	75/75	16/16	22/22	

DU: duodenal ulcer; GC: gastric cancer; GT: gastritis; PUD: peptic ulcer disease; NA: not available; yr: year

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population in Indo-Pacific region who had *H. pylori*-infected gastric disorders were CagA positive. On stratification, Vietnam (90%, 95%CI = 73–99%; 4 studies) had the highest pooled prevalence of CagA, followed by Malaysia (81%, 95%CI = 68–91%; 6 studies), Thailand (81%, 95%CI = 48–99%; 6 studies), Myanmar (90%, 95%CI = 85–93%; 3 studies), and India (60%, 95%CI = 34–84%; 4 studies). Notably, all except the studies in Myanmar (I^2: 0%). showed substantial heterogeneity (I^2: > 93%). There was only a single study from Bhutan (100%, 95% CI = 98–100%), Indonesia (98%, 95%CI = 88–100%) and Singapore (88%, 95%CI = 80–93%).

CagA prevalence in *H. pylori*-infected gastritis

On stratification by type of gastric disorders, CagA prevalence in *H. pylori*-infected gastritis was reported in 23 studies. Overall, the summary prevalence of CagA in gastritis was 79% (95%CI = 67–90%). Vietnam (94%, 95%CI = 90–97%; 2 studies) had the highest pooled prevalence, followed by Malaysia (88%, 95%CI = 69–99%; 6 studies), Thailand (82%, 95%CI = 56–98%; 6 studies), Myanmar (88%, 95%CI = 82–93%; 2 studies), and India (20%, 95%CI = 6–40%; 4 studies). Heterogeneity was substantial among studies in Malaysia and Thailand (I^2: > 96%), but it was absent among studies in India, Myanmar, and Vietnam. Only a single study was
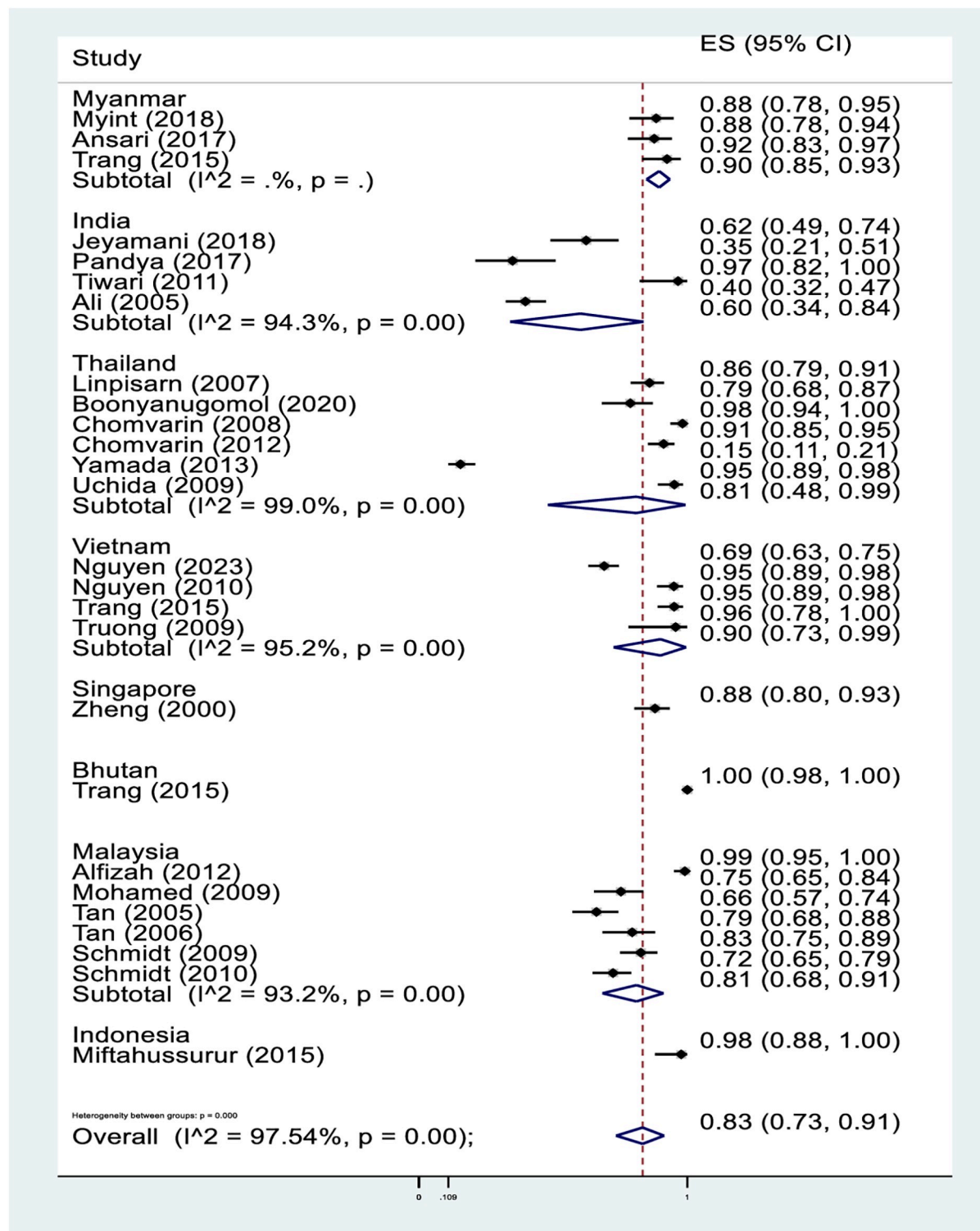
Fig 2. Pooled prevalence of CagA in *H. pylori*-infected gastric disorders. Note: A horizontal line represents individual study. A point estimate (prevalence) of an individual study is represented by the square in the line. The line's width indicates 95% confidence interval. A pooled prevalence is represented by the diamond. Proportion is the estimated percentage of prevalence. The I^2 value in percentage denotes the degree of heterogeneity between studies. For example, a pooled prevalence of CagA in Myanmar is 90% (95%CI:85–93%, I^2:0%).

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available either from Bhutan (100%, 95%CI = 98–100%), Indonesia (98%, 95%CI = 88–100%) or Singapore (90%,95%CI = 77–97%) (S1 Fig).

CagA prevalence in *H. pylori*-infected peptic ulcer disease

Seventeen studies reported data on CagA prevalence among participants with *H. pylori*-infected peptic ulcer disease. Overall, the summary prevalence of CagA in participants with *H. pylori*-infected peptic ulcer disease was 86% (95%CI = 73–96%). The highest summary prevalence of CagA was reported from Thailand 94% (95%CI = 87–99%; 4 studies), followed by Vietnam (91%, 95%CI:50–100%; 4 studies) and Malaysia (86%, 95%CI = 37–100%, 4 studies). India accounted for a prevalence of 59% (95%CI = 43–75%; 4 studies). Heterogeneity was substantial among these studies in India, Malaysia, Thailand, and Vietnam (I^2: >57%). The single studies from Bhutan (100% (95%CI = 91–100%) and Singapore (87%, 95%CI = 76–94%) reported a prevalence of CagA in participants with peptic ulcer disease (S2 Fig).

CagA prevalence in *H. pylori*-infected gastric cancer

A total of 11 studies reported data on CagA prevalence among participants with *H. pylori*-infected gastric cancer. Overall, 83% (95%CI = 51–100%, I^2: 95%) of patients with gastric cancer had CagA positives. On stratification by countries, Malaysia had the highest prevalence of CagA (96%, 95%CI = 87–100%, I^2: 0%; 2 studies), followed by India (90%, 95%CI = 75–99%; I^2: 0%; 2 studies), and Thailand (71%, 95%CI = 16–100%%; I^2: 96%; 5 studies). Notably, heterogeneity was substantial among studies in Thailand ($I^2 > 98\%$), while studies in India and Malaysia had the absence of heterogeneity. A single study was available from Vietnam (92%, 95%CI = 65–99%) (S3 Fig).

Associations between CagA toxin and the risk of gastric disorders

Table 2 provides data on the association studies. Fifteen studies were available for an assessment of the relationship between CagA and gastric disorder [31, 33–37, 41–46, 49, 52, 53]. Of

Table 2. Distribution of CagA in gastric phenotype.

Sr. no.	Study	Year	Country	GT+ (total)	PUD+(total)	GC+(total)
1	Ali [31]	2005	India	4 (56)	49 (104)	12(14)
2	Boonyanugomol [33]	2020	Thailand	38 (50)	17 (20)	8 (18)
3	Chomvarin [34]	2008	Thailand	60 (62)	34 (68)	16 (32)
4	Chomvarin [35]	2012	Thailand	65 (68)	52 (109)	17 (35)
5	Jeyamani [36]	2018	India	21 (42)	12 (14)	NA
6	Linpisarn [37]	2007	Thailand	48 (58)	68 (73)	3 (7)
7	Nguyen [41]	2010	Vietnam	71 (76)	24 (24)	NA
8	Nguyen [42]	2023	Vietnam	NA	185 (268)	NA
9	Pandya [43]	2017	India	9 (34)	3 (12)	NA
10	Schmidt [44]	2009	Malaysia	103 (105)	NA	21 (42)
11	Schmidt [45]	2010	Malaysia	121 (121)	16 (48)	22 (42)
12	Tan [46]	2005	Malaysia	59 (75)	25 (53)	NA
13	Trang [49]	2015	Vietnam	72 (76)	25 (51)	NA
			Bhutan	161(161)	38 (38)	NA
14	Yamada [52]	2013	Thailand	71 (76)	24 (48)	NA
15	Zheng [53]	2000	Singapore	58 (77)	37 (78)	NA

GC: gastric cancer; GT: gastritis; NA: not available/not reported; PUD: peptic ulcer disease.

these, 13, seven, and six studies compared the risk between peptic ulcer disease and gastritis, gastric cancer and gastritis, and peptic ulcer disease and gastric cancer, respectively.

Thirteen studies were available for a comparison of the CagA positive between peptic ulcer disease and gastritis risks [31–37, 41, 43–46, 49, 52, 53]. Only six studies were available for a comparison of the CagA positive between gastric cancer and gastritis [33–35, 37, 44, 45]. Five studies reported data for all three types of gastric disorder [33–35, 37, 44].

Overall, CagA was a comparable risk for both peptic ulcer disease and gastritis (OR 1.03, 95%CI = 0.37 to 2.91, 1763 participants, 13 studies, I^2: 82%). There were no differences in the risks in any subgroup of countries (Fig 3). Overall, presence of CagA was associated with an increased risk of developing gastric cancer than gastritis (OR 2.53, 95%CI = 1.15 to 5.53, 613 participants, 7 studies, I^2: 78%) (Fig 4), but a comparable risk for both gastric cancer and peptic ulcer disease (OR 0.44, 95%CI = 0.08 to 2.34, 404 participants, 6 studies, I^2: 67%) (Fig 5). Notably, in all these comparisons, there were substantial heterogeneities among the studies.

Publication bias

A funnel plot showed asymmetry, indicating the presence of publication bias in comparison between peptic ulcer disease and gastric cancer (13 studies included) (Fig 6). For other comparisons (peptic ulcer diseases vs gastritis; gastric cancer vs gastritis) it was not possible to perform Eggers's test as they had less than the ten studies recommended for this assessment.

Discussion

This study presents information on the pooled prevalence of CagA among those with *H. pylori*-infected gastric disorders based on the data from 24 observational studies across eight countries in the Indo-Pacific region. According to the findings, distinct *H. pylori* CagA strains are circulating with geographical variation. The global prevalence of CagA-positive *H. pylori* ranges from 43% to 90% [54]. and that was comparable with the current findings of 82% in gastric disorders. After recalculation of data provided in a published review including four Asian countries [55], CagA prevalence of 84% (95%CI = 67–100%) was also comparable with the current findings, which encompassed 24 studies from eight Indo-Pacific countries (82%, 95%CI = 72–99%).

Stomach mucosa in the antrum and corpus almost always becomes infiltrated by neutrophilic and mononuclear cells during *H. pylori* colonization [56]. The primary condition associated with *H. pylori* colonization is chronic active gastritis, and additional conditions specifically linked to *H. pylori* are the result of this continuing inflammatory process [5, 56]. Hence, our analysis of the risk of developing peptic ulcer disease or gastric cancer compared to gastritis is meaningful. Compared with gastritis, the presence of CagA had an increased risk of developing gastric cancer observed in this study could be explained with biological plausibility.

The difference in the occurrences of CagA of gastric cancer in India, Thailand, and Malaysia may be explained by the phenomena of various strains circulating within different ethnic groups, in conjunction with host and specific environmental factors. As described elsewhere [57], *H. pylori* may have arrived in Asia as part of the five ancient human migrations. The history of human population expansion in this region has been influenced by multiple migrations and population expansions.

Molecular quantitative analysis showed that the load of CagA injected after one hour was approximately 16% of the total CagA existing in the adjacent bacteria, and 30% after three hours [58]. Considering of the low percentage of the total amount of bacteria in the infection and the load of CagA injected by the bound bacteria, this might be one of many reasons why
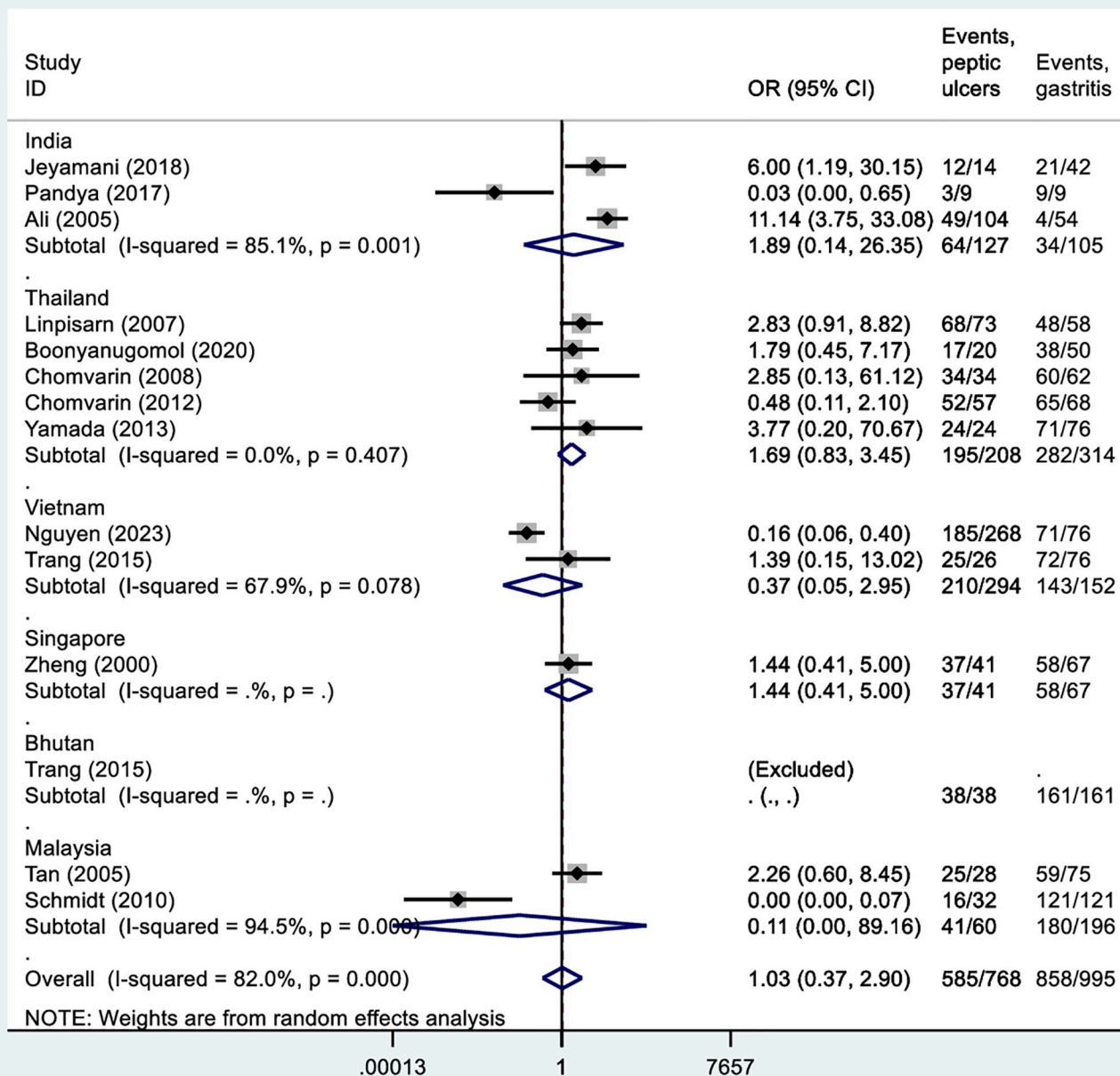
Fig 3. Forest plot indicating the association of CagA in peptic ulcer disease and gastritis.

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there was low occurrence of gastric cancer in the countries with high incidence of *H. pylori* infection.

CagA in the development of gastric disorder is not yet fully understood. There are many ways in which translocated CagA interferes with host cell functions. For instance, the CagA–PAR1 interaction [59], the interaction between SHP2 and gp130 (Src homology 2 domain-containing protein tyrosine phosphatase 2 and glycoprotein 130) [60], the interaction of the IL6/gp130/STAT3 (interleukin-6/glycoprotein 130/signal transducer and activation of
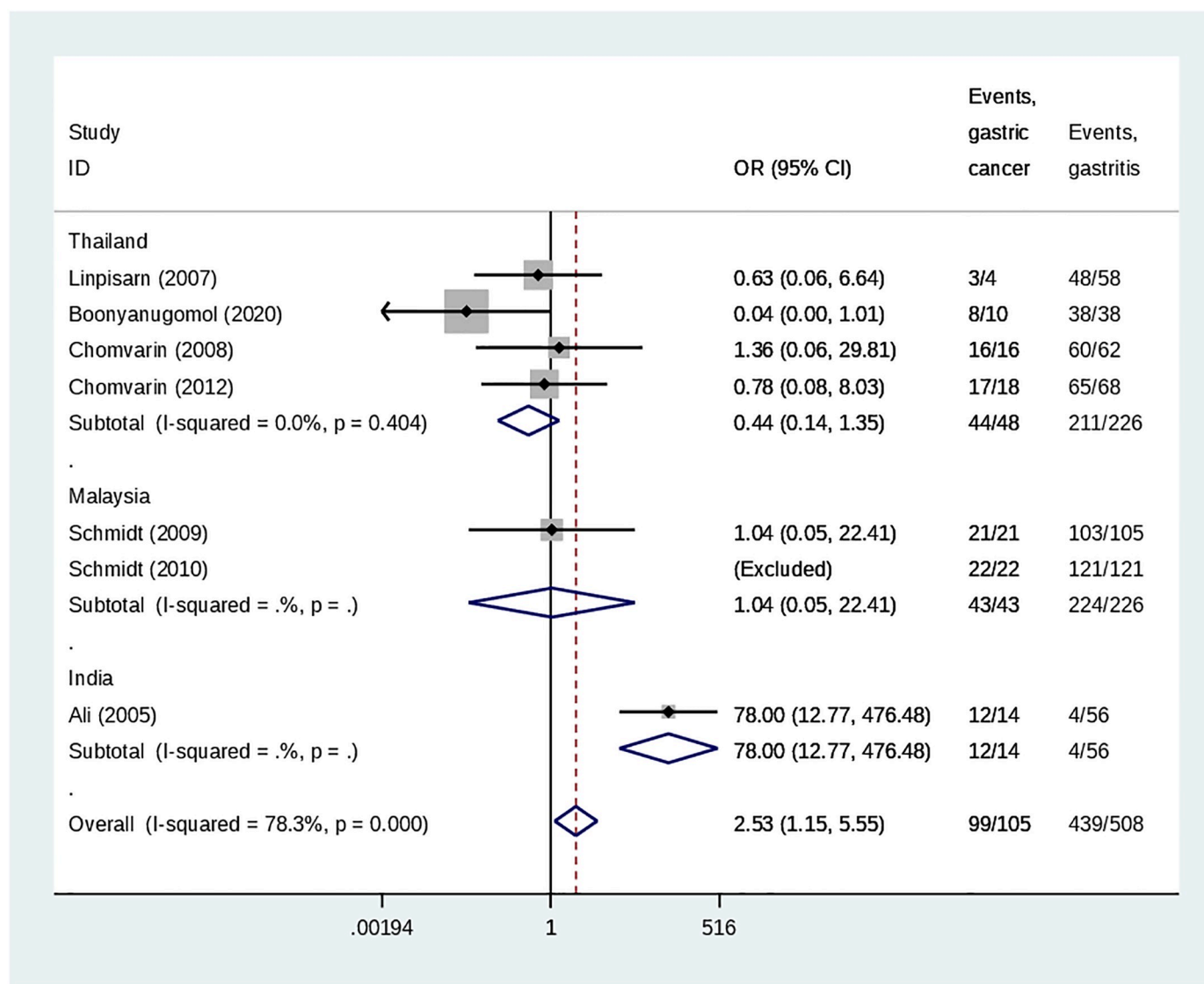
Fig 4. Forest plot indicating the association of CagA in gastric cancer and peptic ulcer disease.

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transcription 3) pathway [60, 61], inflammation attribution to the changes in chemokine profile of tissue [62], among others. Like previous meta-analyses [63, 64], the current study documented that CagA toxin of *H. pylori* may contribute to gastric cancer.

Study limitation

There are some limitations. This study only reviewed published studies written in English. As such, there is an issue of information bias because it is likely to miss unpublished studies or the non-English publications that may have been appropriate to include. Regarding an inherent limitation of cross-sectional/survey studies, the prevalence estimates in the primary studies identified for the present analysis could vary as times passes. Considering the associations observed in the current meta-analysis, it would be crucial to substantiate the results using cohort studies with extended follow-up times. The merit of observational studies is that they
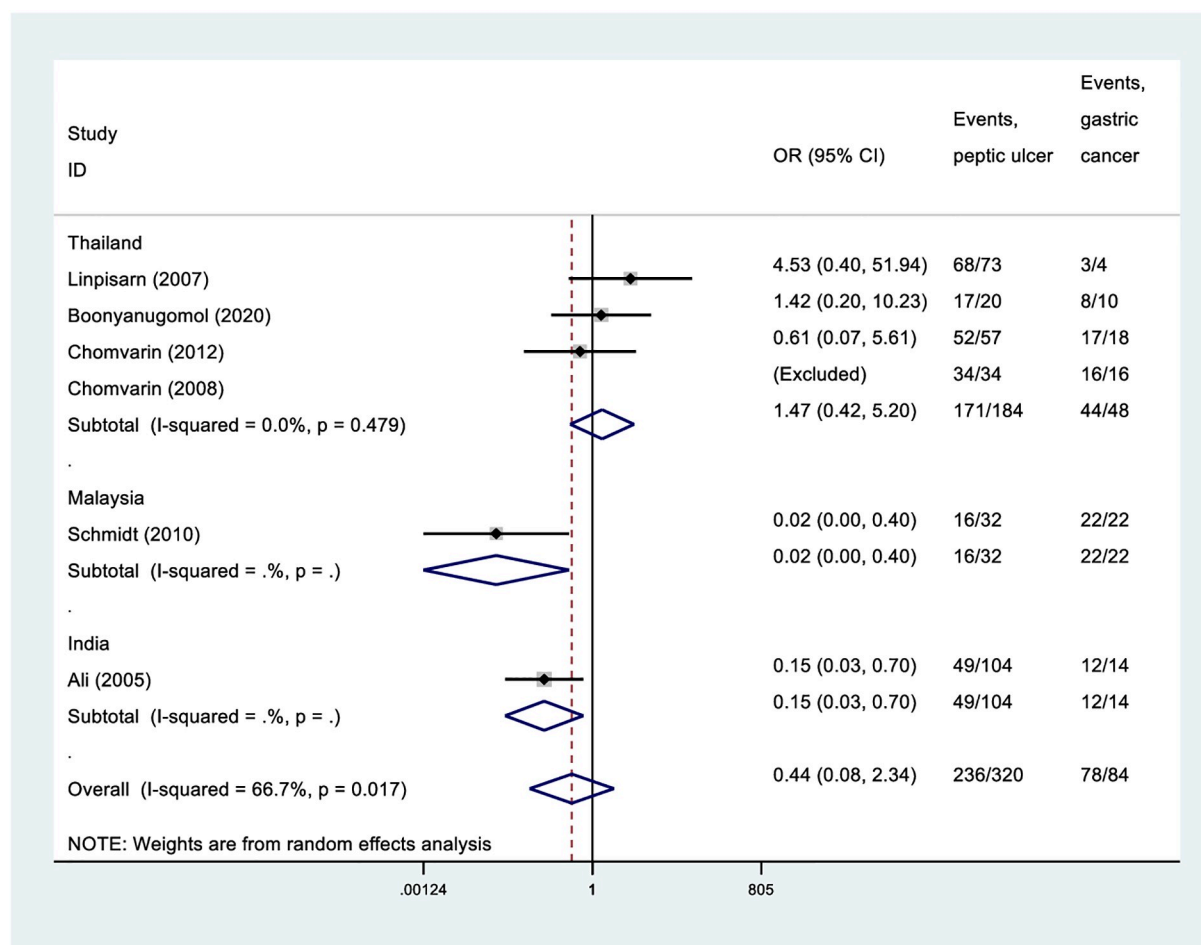
Fig 5. Forest plot indicating the association of CagA with gastric cancer and peptic ulcer disease.

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can determine health problems existing, reflecting real-life conditions. Due to the paucity of data, subgroups such as the age of the study population, and unique types of gastric cancer (e.g., intestinal, cardina, diffuse) could not be analyzed. The present study included single genotype CagA. Hence, selection bias is a concern. Future studies should consider multiple genotypes in analysis. Due to publication bias related to small studies that tended to exaggerate the true effect [65], as well as limited number of studies that could compare CagA between the risks of gastric cancer and peptic ulcer disease, a type II error may be an issue. The estimated prevalence in this study with substantial heterogeneity might not be related to sampling error only [66]. High heterogeneity may be observed albeit with more homogenous subgroups such as countries. Therefore, a high I^2 value is not a signal that the data are inconsistent [25]. Thus, these cautions should be considered while interpreting the findings.

Implications

The higher incidence of CagA in peptic ulcer disease suggests any *H. pylori* infection prevention program can help preventing gastritis from progressing to peptic ulcers, and gastric cancer a more costly and potentially fatal result of a common and treatable bacterial infection. Additionally, a higher risk of stomach cancer affects the capacity to access efficient treatments
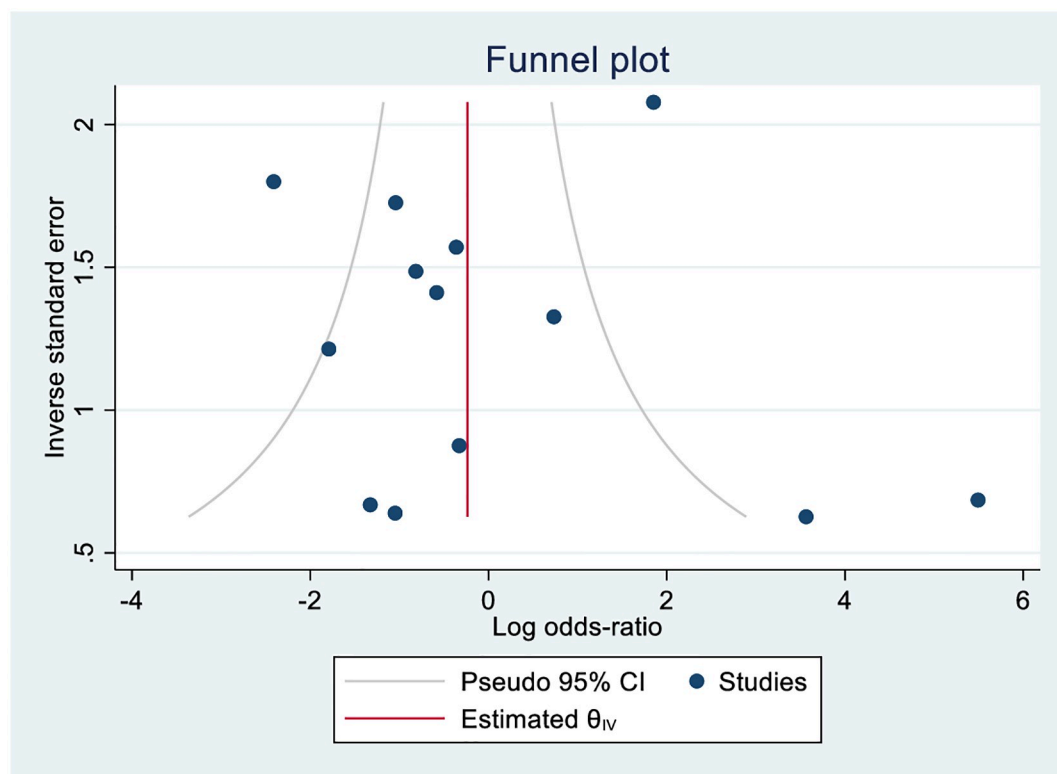
Fig 6. Funnel plot asymmetry showing publication bias.

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that could lower the risk of stomach cancer. Given that different countries have distinct risks that reflect differences in their ethnic composition, identifying and prioritizing the high-risk population will help with better planning and prioritization of health programs to manage this cluster of disorders associated with *H. pylori* and may also make services more accessible and affordable. Reiterating the substantial evidence of association between a treatable infection and a high burden of disease and health care utilisation can help improve equity in their programs during this second half of the Sustainable Development Goal period, when countries in the region are struggling to attain the universal health coverage goal set [20].

Conclusion

Findings suggest that the distribution of CagA in *H. pylori*-infected gastric disorders varies among gastric disorders in the study countries, and CagA has a plausible role in the development of gastric cancer. It is important to the quality of care for the management of gastric diseases, particularly in a region where the prevalence of these disorders is high. Better strategies for effective treatment for high-risk groups are required for health programs to revisit this often-neglected infectious disease.

Supporting information

S1 Checklist. PRISMA-2020 checklist.
(DOC)

S1 Table. Search strategy.
(DOC)

S2 Table. Excluded studies and the reasons for exclusion.
(DOC)

S3 Table. Quality assessment with the NOS assessment criteria.
(DOC)

S1 Fig. Pooled prevalence of CagA in *H. pylori*- infected gastritis.
(DOC)

S2 Fig. Pooled prevalence of CagA in *H. pylori*- infected peptic ulcer disease.
(DOC)

S3 Fig. Pooled prevalence of CagA in *H. pylori*- infected gastric cancer.
(DOC)

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Author Contributions

Conceptualization: Yong Poovorawan, Maxine A. Whittaker.

Data curation: Cho Naing, Htar Htar Aung, Saint Nway Aye.

Formal analysis: Cho Naing, Htar Htar Aung, Saint Nway Aye.

Methodology: Cho Naing, Htar Htar Aung, Yong Poovorawan, Maxine A. Whittaker.

Supervision: Maxine A. Whittaker.

Writing – original draft: Cho Naing, Htar Htar Aung.

Writing – review & editing: Cho Naing, Maxine A. Whittaker.

References

1. Chmiela M, Karwowska Z, Gonciarz W, Allushi B, Stączek P. Host pathogen interactions in Helicobacter pylori related gastric cancer. World J Gastroenterol. 2017: 23, 1521–1540. https://doi.org/10.3748/wjg.v23.i9.1521 PMID: 28321154

2. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet.1984; 1:1311–1315. https://doi.org/10.1016/s0140-6736(84)91816-6 PMID: 6145023

3. IARC. Anonymous live flukes and *Helicobacter pylori*. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Lyon, 7–14 June 1994. IARC Monogr. Eval. Carcinog. Risks Hum.1994; 61:1–241.

4. Abadi ATB. Strategies used by *helicobacter pylori* to establish persistent infection. World J Gastroenterol. 2017; 23(16):2870–2882.

5. Israel DA, Peek RM Jr. The role of persistence in *Helicobacter pylori* pathogenesis. Curr Opin Gastroenterol. 2006; 22(1):3–7.

6. Bakhti SZ, Latifi-Navid S, Safaralizadeh R. *Helicobacter pylori*-related risk predictors of gastric cancer: The latest models, challenges, and future prospects. Cancer Med. 2020; 9(13):4808–4822.

7. Salama N, Guillemin K, McDaniel TK, Sherlock G, Tompkins L, Falkow S. A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. Proc Natl Acad Sci. 2000; 97:14668–14673.

8. Selbach M, Moese S, Hauck CR, Meyer TF, Backert S. Src is the kinase of the *Helicobacter pylori CagA* protein *in vitro* and *in vivo*. J Biol Chem. 2002; 277: 6775–6778.

9. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, et al. *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. U.S.A.1996: 93: 14648–14653.

10. Backert S, Tegtmeyer N, Selbach M. The versatility of *Helicobacter* pylori CagA effector protein functions: The master key hypothesis. Helicobacter. 2010; 15:163–176.

11. Kwok T, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, et al. Helicobacter exploits integrin for type IV secretion and kinase activation. Nature. 2007: 49:862–6. https://doi.org/10.1038/nature06187 PMID: 17943123

12. Tegtmeyer N, Wessler S, Backert S. Role of the cag-pathogenicity island encoded type IV secretion system in *Helicobacter pylori* pathogenesis. FEBS J. 2011: 278:1190–1202.

13. Yong X, Tang B, Li BS, Xie R, Hu CJ, Luo G et al. *Helicobacter pylori* virulence factor *Cag*A promotes tumorigenesis of gastric cancer via multiple signaling pathways. Cell Commun Signal. 2015: 13:30.

14. Higashi HA, Nakaya R, Tsutsumi K, Yokoyama K, Fujii Y, Ishikawa S, et al. *Helicobacter pylori Cag*A induces Ras-independent morphogenetic response through SHP-2 recruitment and activation, J Biol Chem. 2004: 79:17205–17216.

15. Miwa H, Go MF, Sato N. *H. pylori* and gastric cancer: the Asian enigma. Am J Gastroenterol.2002: 97 (5):1106–1112.

16. Matsukura N, Yamada S, Kato S, Tomtitchong P, Tajiri T, Miki M, et al. Genetic differences in interleukin-1 beta polymorphisms among four Asian populations: an analysis of the Asian paradox between *H. pylori* infection and gastric cancer incidence. J Exp Clin Cancer Res. 2003: 22:47–55.

17. GLOBOCAN: 50-bangladesh-fact-sheets.pdf (iarc.fr) gco.iarc.fr/today/data/factsheets/populations/50-bangladesh-fact-sheets.pdf 2020

18. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. Gastroenterology. 2017: 153:420–429.

19. Wang R, Li Z, Liu S, Zhang D. Global, regional, and national burden of 10 digestive diseases in 204 countries and territories from 1990 to 2019. Front Public Health. 2023; 11:1061453. https://doi.org/10.3389/fpubh.2023.1061453 PMID: 37056655

20. Abbasi-Kangevari M, Ahmadi N, Fattahi N, Rezaei N, Malekpour MR, Ghamari SH, et al Quality of care of peptic ulcer disease worldwide: A systematic analysis for the global burden of disease study 1990–2019. PLoS One. 2022; 17(8): e0271284. https://doi.org/10.1371/journal.pone.0271284 PMID: 35913985

21. Bailey KS, Brown HE, Lekic V, Pradeep K, Merchant JL, Harris RB. *Helicobacter pylori* treatment knowledge, access and barriers: A cross-sectional study. Helicobacter. 2023; 28(2):e12954. https://doi.org/10.1111/hel.12954 PMID: 36748455

22. Page MJ, McKenzie J E, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021; 372, n71. https://doi.org/10.1136/bmj.n71 PMID: 33782057

23. Zheng TH, Zhao JL, Guleng B. Advances in molecular biomarkers for gastric cancer. Crit Rev Eukaryot Gene Expr.2015: 25:299–305. https://doi.org/10.1615/critreveukaryotgeneexpr.2015014360 PMID: 26559090

24. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al: The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2013. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp,

25. Barker TH, Migliavaca CB, Stein C, Colpani V, Falavigna M, Aromataris E, et al. Conducting proportional meta-analysis in different types of systematic reviews: a guide for synthesisers of evidence. BMC Med Res Methodol.2021; 21, 189. https://doi.org/10.1186/s12874-021-01381-z PMID: 34544368

26. Nyaga VN, Arbyn M, Aerts M. METAPROP_ONE: Stata module to perform fixed and random effects meta-analysis of proportions. Statistical Software Components S457861, Boston College Department of Economics, revised 26 Jan 2017.

27. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin. Trials. 1986; 7, 177–188. https://doi.org/10.1016/0197-2456(86)90046-2 PMID: 3802833

28. Deeks JJ, Higgins JP, Altman DG, editor(s). Chapter 10: Analysing data and undertaking meta-analyses. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane

Handbook for Systematic Reviews of Interventions Version 6.3 (updated February 2022). Cochrane, 2022. training.cochrane.org/handbook.

29. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997: 315:629–364. https://doi.org/10.1136/bmj.315.7109.629 PMID: 9310563

30. Alfizah H, Ramelah M, Rizal AM, Anwar AS, Isa MR. Association of Malaysian *Helicobacter pylori* virulence polymorphisms with severity of gastritis and patients' ethnicity. Helicobacter. 2012; 17:340–349. https://doi.org/10.1111/j.1523-5378.2012.00956.x PMID: 22967117

31. Ali M, Khan AA, Tiwari SK, Ahmed N, Rao LV, Habibullah CM. Association between cag-pathogenicity island in *Helicobacter pylori* isolates from peptic ulcer, gastric carcinoma, and non-ulcer dyspepsia subjects with histological changes. World J Gastroenterol 2005; 11(43):6815–6822.

32. Ansari S, Kabamba ET, Shrestha PK, Aftab H, Myint T, Tshering L, et al. *Helicobacter pylori* bab characterization in clinical isolates from Bhutan, Myanmar, Nepal and Bangladesh. PLoS One. 2017; 12: e0187225.

33. Boonyanugomol W, Kongkasame W, Palittapongarnpim P, Baik SC, Jung MH, Shin MK, et al. Genetic variation in the cag pathogenicity island of *Helicobacter pylori* strains detected from gastroduodenal patients in Thailand. Braz J Microbiol. 2020; 51:1093–1101.

34. Chomvarin C, Namwat W, Chaicumpar K, Mairiang P, Sangchan A, Sripa B, et al. Prevalence of *Helicobacter pylori* vacA, cagA, cagE, iceA and babA2 genotypes in Thai dyspeptic patients. Int J Infect Dis.2008; 12: 30–36.

35. Chomvarin C, Phusrim K, Sawadpanich K, Mairiang P, Namwat W, Wongkham C, et al. Prevalence of cagA EPIYA motifs in *Helicobacter pylori* among dyspeptic patients in northeast. Southeast Asian J Trop Med Public Health. 2012; 43:105–115.

36. Jeyamani L, Jayarajan J, Leelakrishnan V, Swaminathan M. *CagA* and *VacA* genes of *Helicobacter pylori* and their clinical relevance. Indian J Pathol Microbiol. 2018; 61(1): 66–69.

37. Linpisarn S, Suwan W, Lertprasertsuk N. *Helicobacter pylori* cagA, vacA and iceA genotypes in northern Thai patients with gastric disease. Southeast Asian J Trop Med Public Health. 2007; 38(2):356–362.

38. Miftahussurur M, Syam AF, Makmun D, Nusi IA, Zein LH, Zulkhairi, et al. *Helicobacter pylori* virulence genes in the five largest islands of Indonesia. Gut Pathog. 2015; 7:26.

39. Mohamed R, Hanafiah A, Rose IM, Manaf MR, Abdullah SA, Sagap I, et al. *Helicobacter pylori* cagA gene variants in Malaysians of different ethnicity. Eur J Clin Microbiol Infect Dis. 2009; 28:865–869.

40. Myint T, Miftahussurur M, Vilaichone RK, Ni N, Aye TT, Subsomwong P, et al. Characterizing *Helicobacter pylori cagA* in Myanmar. Gut Liver. 2018; 12:51–57.

41. Nguyen TL, Uchida T, Tsukamoto Y, Trinh DT, Ta L, Mai BH, et al. *Helicobacter pylori* infection and gastroduodenal diseases in Vietnam: a cross-sectional, hospital-based study. BMC Gastroenterol.2010; 10:114

42. Nguyen TC, Tang NLC, Le GKN, Nguyen VT, Nguyen KHG, Che TH, et al. *Helicobacter pylori* infection and peptic ulcer disease in symptomatic children in southern Vietnam: a prospective multicenter study. Healthcare.2023; 11, 1658.

43. Pandya HB, Agravat HH, Patel JS. Prevalence of Specific *Helicobacter pylori cagA*, *vacA*, *iceA*, *ureC* genotypes and its clinical relevance in the patients with acid-peptic diseases. J Clin Diagn Res.2017; 11(8): DC23–DC26.

44. Schmidt HM, Goh KL, Fock KM, Hilmi I, Dhamodaran S, Forman D, et al. Distinct cagA EPIYA motifs are associated with ethnic diversity in Malaysia and Singapore. Helicobacter. 2009; 14:256–263. https://doi.org/10.1111/j.1523-5378.2009.00684.x PMID: 19674129

45. Schmidt HM, Andres S, Nilsson C, Kovach Z, Kaakoush NO, Engstrand L, et al. The cagA PAI is intact and functional but hp0520 varies significantly in *Helicobacter pylori* isolates from Malaysia and Singapore. Eur J Clin Microbiol Infect Dis.2010; 29:439–451.

46. Tan HJ, Rizal AM, Rosmadi MY, Goh KL. Distribution of *Helicobacter pylori* cagA, cagE and vacA in different ethnic groups in Kuala Lumpur, Malaysia. J Gastroenterol Hepatol. 2005; 20:589–594.

47. Tan HJ, Rizal AM, Rosmadi MY, Goh KL. Role of *Helicobacter pylori* virulence factor and genotypes in non-ulcer dyspepsia. J Gastroenterol Hepatol.2006; 21: 110–111.

48. Tiwari SK, Sharma V, Sharma VK, Gopi M, Saikant R, Nandan A, et al. Phylogenetic analysis, based on EPIYA repeats in the CagA gene of Indian *Helicobacter pylori*, and the implications of sequence variation in tyrosine phosphorylation motifs on determining the clinical outcome. Genet Mol Biol. 2011; 34: 280–285.

49. Trang TT, Shiota S, Matsuda M, Binh TT, Suzuki R, Vilaichone RK, et al. The prevalence of *Helicobacter pylori* virulence factors in Bhutan, Vietnam, and Myanmar is related to gastric cancer incidence. Biomed Res Int. 2015.

50. Truong BX, Mai VT, Tanaka H, Ly le T, Thong TM, Hai HH, et al. Diverse characteristics of the cagA gene of *Helicobacter pylori* strains collected from patients from South Vietnam with gastric cancer and peptic ulcer. J Clin Microbiol. 2009; 47: 4021–4028.

51. Uchida T, Nguyen LT, Takayama A, Okimoto T, Kodama M, Murakami K, et al. Analysis of virulence factors of *Helicobacter pylori* isolated from a Vietnamese population. BMC Microbiol. 2009; 9:175.

52. Yamada S, Kato S, Matsuhisa T, Makonkawkeyoon L, Yoshida M, Chakrabandhu T, et al. Predominant mucosal IL-8 mRNA expression in non-cagA Thais is risk for gastric cancer. World J Gastroenterol. 2013; 19(19):2941–2949. https://doi.org/10.3748/wjg.v19.i19.2941 PMID: 23704827

53. Zheng PY, Hua J, Yeoh KG, Ho B. Association of peptic ulcer with increased expression of Lewis antigens but not cagA, iceA, and vacA in *Helicobacter pylori* isolates in an Asian population. Gut.2000; 47:18–22.

54. Li Y, Choi H, Leung K, Jiang F, Graham DY, Leung WK. Global prevalence of *Helicobacter pylori* infection between 1980 and 2022: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2023; 8(6):553–564. https://doi.org/10.1016/S2468-1253(23)00070-5 PMID: 37086739

55. Sahara S, Sugimoto M, Vilaichone RK, Mahachai V, Miyajima H, Furuta T, et al. Role of *Helicobacter pylori* cagA EPIYA motif and vacA genotypes for the development of gastrointestinal diseases in Southeast Asian countries: a meta-analysis. BMC Infect Dis. 2012; 12:223.

56. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev.2006; 19:449–490.

57. Breurec S, Guillard B, Hem S, Brisse S, Dieye FB, Huerre M, et al. Evolutionary History of *Helicobacter pylori* Sequences reflect past human migrations in Southeast Asia. Plos One.2011; 6(7): e22058.

58. Jimenez-Soto LF, Haas R. The CagA toxin of *Helicobacter pylori*: abundant production but relatively low amount translocated. Sci Rep. 2016; 6:23227

59. Saadat I, Higashi H, Obuse C, Umeda M, Murata-Kamiya N, Saito Y, et al. *Helicobacter pylori* CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. Nature.2007; 447, 330–333.

60. Lee IO, Kim JH, Choi YJ, Pillinger MH, Kim SY, Blaser MJ, et al. *Helicobacter pylori* CagA phosphorylation status determines the gp130-activated SHP2/ERK and JAK/STAT signal transduction pathways in gastric epithelial cells. J Biol Chem. 2010; 285:16042–16050.

61. Ohtani T, Ishihara K, Atsumi T, Nishida K, Kaneko Y, Miyata T, et al. Dissection of signaling cascades through gp130 in vivo: reciprocal roles for STAT3- and SHP2-mediated signals in immune responses. Immunity. 2000; 12:95–105. https://doi.org/10.1016/s1074-7613(00)80162-4 PMID: 10661409

62. Posselt G, Backert S, Wessler S. The functional interplay of *Helicobacter pylori* factors with gastric epithelial cells induces a multi-step process in pathogenesis. Cell Commun Signal. 2013; 11:77.

63. Matos JI, de Sousa HA, Marcos-Pinto R, Dinis-Ribeiro M. *Helicobacter pylori CagA* and *VacA* genotypes and gastric phenotype: a meta-analysis. Eur J Gastroenterol Hepatol. 2013; 25:1431–1441.

64. Pormohammad A, Mohtavinejad N, Gholizadeh P, Akrami S, Asadi A, Kazemian H, et al. Global estimate of gastric cancer in Helicobacter pylori-infected population: A systematic review and meta-analysis. J Cell Physiol. 2019; 234:1208–1218. https://doi.org/10.1002/jcp.27114 PMID: 30132888

65. Newcombe RG. Towards a reduction in publication bias. Br Med J (Clin Res Ed). 1987; 295 (6599):656–659 https://doi.org/10.1136/bmj.295.6599.656 PMID: 3117278

66. Barendregt JJ, Doi SA, Lee YY, Norman RE, Vos T. Meta-analysis of prevalence. J. Epidemiol. Community Health.2013; 67, 974–978. https://doi.org/10.1136/jech-2013-203104 PMID: 23963506