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**Relationship between *Helicobacter pylori* infection and programmed death-ligand 1
in gastric cancer: A meta-analysis**

Yang HC *et al.* *H. pylori* and PD-L1 in GC

Abstract

BACKGROUND

Gastric cancer (GC) is one of the most common malignancies worldwide, and *Helicobacter pylori* (HP) infection is a well-established risk factor for its development. Programmed death-ligand 1 (PD-L1) expression is a crucial biomarker for predicting the efficacy of immune checkpoint inhibitors in cancer treatment. While HP infection and PD-L1 expression in GC may be linked, the relationship between them remains unclear, in part because there have been conflicting results reported from various studies.

AIM

To perform a meta-analysis to assess the relationship between HP and PD-L1 expression in patients with GC.

METHODS

A systematic literature review was conducted using PubMed, Embase, Cochrane Library, and Web of Science databases. Observational studies that examined the association between HP infection and PD-L1 expression in patients with GC were included. Odds ratios and 95% confidence intervals were calculated to estimate the association. Heterogeneity was assessed using Cochrane's Q test and I^2 statistic. A random-effects model was used due to significant heterogeneity across studies.

RESULTS

Fourteen studies involving a total of 3069 patients with GC were included. The pooled analysis showed a significant association between HP infection and increased PD-L1 expression in GC tissues (odd ratio = 1.69, 95% confidence interval: 1.24-2.29, $P < 0.001$, $I^2 = 59\%$). Sensitivity analyses confirmed the robustness of these findings. Subgroup analyses did not show significant variation based on geographic region, sample size, or

method of PD-L1 assessment. Publication bias was minimal, as shown by funnel plots and Egger's regression test.

CONCLUSION

HP infection is associated with increased PD-L1 expression in GC, suggesting that HP status may influence the response to programmed cell death protein 1/PD-L1 blockade therapy.

Key Words: *Helicobacter pylori*; Gastric cancer; Programmed cell death protein 1/programmed death-ligand 1; Immune checkpoint blockade therapy; Pathogenesis

Yang HC, Fu CF, Qiao LJ, Long GH, Yang LF, Yao B. Relationship between *Helicobacter pylori* infection and programmed death-ligand 1 in gastric cancer: A meta-analysis. *World J Clin Oncol* 2024; In press

INTRODUCTION

Gastric cancer (GC) is among the top five most frequently occurring malignant tumors on a global scale and is the fourth major reason for deaths related to cancer. In 2022, there were approximately 960000 new cases of GC globally and about 650000 deaths[1-3]. Although surgery and postoperative adjuvant radiochemotherapy are considered the main treatment methods for patients with early-stage GC, many are diagnosed at an advanced stage of cancer, limiting the benefits of the aforementioned treatments and patient survival. The continued development of immune checkpoint blocking therapy, such as programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitors, has changed treatment approaches for GC, showing significant effects either as monotherapy or in combination with chemotherapy[4,5].

PD-L1 is a type I transmembrane protein that inhibits immune responses through its interaction with its receptor PD-1, which is expressed on activated T and B cells and other immune cells[6]. Therefore, PD-L1 upregulation in tumors allows them to evade

immune surveillance by inhibiting immune cell activation. In contrast to traditional treatments that target cancer cells directly, PD-1/PD-L1 inhibitors reactivate the patient's immune system for neoplasm treatment[7]. High PD-L1 expression is not only associated with reduced overall survival (OS) in GC but is also a strong predictive marker for the response of GC patients to immunotherapy[8,9]. In summary, it is important to test for PD-L1 in GC tissue for prognostic assessment and selecting immunotherapy.

Approximately 50% of the world's population is infected with *Helicobacter pylori* (HP)[10], which is classified as a group 1 carcinogen by the World Health Organization[11], and over 95% of patients with GC have a history of HP infection[12]. Although most individuals infected with HP are asymptomatic, long-term HP infection can lead to chronic gastritis, producing reactive oxygen species that may cause DNA damage, thereby initiating a cancer cascade reaction[13]. Meta-analyses have shown that GC patients infected with HP may have a longer OS rate compared to uninfected patients[14,15]. *In vitro* and *in vivo* studies have demonstrated that HP infection may increase the expression of PD-L1 in gastric tissue[16,17], which indicates a potential correlation between HP infection and PD-L1 expression in GC. However, other studies have demonstrated that HP does not increase the risk of PD-L1 expression in GC tissue[18]. Therefore, we performed a systematic analysis to figure out whether HP infection is related to PD-L1 expression in GC patients. The findings from these studies may help us understand the potential interaction between HP infection and the efficacy of immunotherapy in patients with GC.

MATERIALS AND METHODS

This study adhered to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement[19,20] and the Cochrane Handbook[21] for the conception, conduct, and reporting of the study. The study report conforms to the broad EQUATOR guidelines[22].

Database search

Electronic databases, including PubMed, Embase, Cochrane Library, and Web of Science, were searched from inception to June 30, 2024 using a combination of search terms related to the following: (1) “*Helicobacter pylori*” or “*H. pylori*” or “HP”; (2) “gastric” or “stomach” or “gastroesophageal junction”; (3) “cancer” or “tumor” or “carcinoma” or “neoplasm”; and (4) “PD-1” or “PD-L1” or “programmed death”. The search was restricted to human studies, and no language restrictions were applied. The reference lists of relevant original and review articles were also manually screened for potential relevant studies.

Study inclusion and exclusion criteria

In accordance with the objectives of the meta-analysis, we established inclusion criteria and adopted the recommended PICOS criteria: P (Patients): Adult patients diagnosed with GC; I (Exposure): Patients infected with HP; C (Control): Patients not infected with HP. The method of verifying HP infection is consistent with the method used in the original study; O (Outcome): Comparing the number of patients with positive PD-L1 expression between HP-infected and uninfected patients. The method and criteria for defining tumor PD-L1 expression are consistent with those applied in the included studies; and S (Study design): Observational studies, such as cross-sectional studies, case-control studies, and cohort studies. Exclude review articles, preclinical studies, studies including other malignant tumor patients, studies not assessing HP infection, or studies not reporting the results of interest. Through these detailed inclusion and exclusion criteria, researchers can ensure that the studies included in the meta-analysis are consistent and comparable in design, execution, and results. This helps to improve the reliability and validity of the results of the meta-analysis and provides a solid evidence base for clinical decision-making and future research.

Data collection and quality assessment

Literature search, data collection, and study quality assessment were independently conducted by two authors. In the case of disagreement, a third author was contacted for discussion and consensus. We collected the following data from each study that were included: Study information, patient demographics, cancer staging and treatment, HP infection diagnostic methods, and tumor PD-L1 expression. The quality of the studies was assessed using the Newcastle-Ottawa scale[23], which scores participant selection criteria, comparability between groups, and outcome validity. The scale scores range from 1 to 9 stars, with more stars indicating higher study quality.

Statistical analysis

The numbers of HP-positive and HP-negative patients with GC whose tumors expressed PD-L1 were extracted from each study. The association between HP infection and tumor PD-L1 expression was presented as odds ratios (ORs) and their corresponding 95% confidence intervals (CIs). Cochrane's Q test and I^2 statistic were used to assess heterogeneity among studies, with $I^2 > 50\%$ reflecting significant heterogeneity[24]. A random-effects model was used to combine the results, taking into account the impact of heterogeneity[21]. Sensitivity analysis was performed by excluding one dataset at a time to assess the impact of individual studies on the meta-analysis results[25]. Publication bias was estimated visually by constructing a funnel plot and supplemented by Egger's regression asymmetry test[26]. Data analysis was performed using R.

RESULTS

Search results

Figure 1 presents the flowchart of our process of searching for literature and conducting studies inclusion process. We obtained 1349 records through the database search and then eliminated 298 duplicate ones. After screening by title and abstract, we excluded an additional 1007 studies, mainly because they didn't align with the objectives of the meta-analysis. Eventually, we carefully examined the full texts of the remaining 44

studies and removed 30 of them for the reasons detailed in Figure 1. Therefore, we included a total of 14 studies for meta-analysis. Therefore, we included a total of 14 studies for meta-analysis (Table 1).

Study characteristics

Overall, 14 studies included a total of 3069 patients with GC in this meta-analysis[17,18,27-38]. These studies were all cross-sectional studies, published between 2015 and 2023, conducted in China, South Korea, Japan, Jordan, the United States, Italy, and Germany. All studies included GC patients, most of whom underwent surgical resection. In eight studies[27,28,33-38], HP infection was detected by histological immunohistochemistry (IHC); in two studies[29,30], 13C breath tests, serological tests, and tissue IHC were used to detect HP infection; in two studies[18,31], 13C breath tests, fecal antigen detection methods, and tissue IHC were used to detect HP infection; in 1 study[32], serological tests were used to detect HP infection; and in 1 study[17], 13C breath tests were used to detect HP infection. The overall prevalence of HP infection was 34.7% (1066/3069). Among all included studies, 6 studies[27,28,30,32,34,35] assessed tumor PD-L1 expression by IHC, with one study defining PD-L1 positivity as $\geq 10\%$ positive cells[27], and the other five studies defining PD-L1 positivity as $\geq 5\%$ positive cells[28,30,32,34,35]. Seven studies assessed tumor PD-L1 expression by combined positive score ($\geq 1\%$)[17,29,31,33,36-38], while one study did not define PD-L1 positivity[18]. Overall, out of all the patients with GC, 41.7% (1279 patients precisely) exhibited positive PD-L1 expression. The Newcastle-Ottawa scale scores of the included studies were consistently within the range of six to seven, which signified that the quality of these studies was at a medium or even better level (Table 2).

Meta-analysis conclusions

Utilizing a random-effects model to conduct a pooled analysis, it was revealed that HP infection exhibited a statistically significant correlation with tumor PD-L1 expression among patients with GC (OR = 1.69, 95%CI: 1.24-2.29, $P < 0.001$; $I^2 = 59\%$; Figure 2). For

the sensitivity analysis, wherein each dataset was sequentially excluded, the outcomes remained consistent (OR = 1.24-2.19, $P < 0.01$; Figure 3). In the subgroup analysis, it was demonstrated that the relationship between HP infection and PD-L1 expression in GC was not substantially influenced by factors such as the country of origin, sample size, prevalence rate of HP infection, PD-L1 assessment methodology, or quality assessment score (all subgroup analyses, $P > 0.05$; Figure 4).

Publication bias

Figure 3 depicts the funnel plot for the meta-analysis of the link between HP infection and PD-L1 expression in GC patients' neoplasms. Visual inspection reveals symmetry, and the Egger's test shows a low probability of publication bias, bolstering the credibility of the analysis (Figure 5).

DISCUSSION

Our meta-analysis of 14 observational studies showed that HP infection in patients with GC is associated with PD-L1 tumor expression. The finding was consistently validated through sensitivity analysis where one dataset was excluded at a time, and through subgroup analysis based on a variety of study characteristics, namely the study country, sample size, HP infection rate, PD-L1 assessment methodology, and quality assessment score. Overall, the results of the meta-analysis show that HP infection is linked to the tumor expression of PD-L1 in GC. These findings are consistent with previous meta-analysis results[39]. Immunotherapy provides survival benefits for cancer patients, but not all patients benefit. PD-L1 expression is an important biological marker for testing whether patients will respond to cancer immunotherapy[42]. Therefore, it is of high importance to determine whether HP infection status affects the efficacy of PD-1/PD-L1 blockade therapy in GC patients. Previous reports have suggested that HP infection may upregulate PD-L1 expression in GC[16,17], but contrary findings have also been reported[18]. Therefore, it is particularly important to use meta-analysis methods to assess the relationship between HP infection and PD-L1 expression in patients with GC.

HP is the first pathogenic factor for GC[11], and treatment to eradicate HP can significantly reduce the incidence of GC by 43%[41]. Therefore, it is conceivable that HP eradication should extend the survival of GC patients, but there are conflicting reports. Kim *et al*[42] reported that HP eradication did not extend the survival of GC patients. In contrast, Zhao *et al*[43] reported that patients receiving anti-HP treatment had a significant advantage in OS and disease-free survival compared to patients not receiving HP treatment. After propensity score matching, the advantage in OS and disease-free survival still existed. There may be two reasons for the different conclusions[15,44-47]: The gene expression profile of tumor tissue is distinct in HP-negative GC cases *vs* HP-positive GC cases after eradication treatment, and the genes that show differential expression are involved in cancer-related signaling pathways, namely the extracellular signal-regulated kinase/mitogen-activated protein kinase and Wnt/ β -catenin signaling pathways. These transcriptional changes could be due to epigenetic changes and chronic inflammation caused by HP infection. In addition, HP bacterial virulence factors, for example CagA and VacA, not only promote bacterial colonization in the gastric mucosa but also induce innate and adaptive immune responses, activate helper T cells 1, and are associated with good prognosis of GC. Taking the relationship between HP and enhanced PD-L1 in GC derived from the meta-analysis can be translated to clinical observations, namely, whether the status of HP infection in GC patients correlates to better responses to immunotherapy. Surprisingly, the opposite has been reported. Che *et al*[18] showed that OS and progression-free survival (PFS) of the HP-negative group are longer than those of the positive group [mOS 17.5 months *vs* 6.2 months, hazard ratio (HR) = 2.85, 95%CI: 1.74-1.78, $P = 0.021$; mPFS 8.4 months *vs* 2.7 months, HR = 3.11, 95%CI: 1.96-5.07, $P = 0.008$]. Multivariate analysis revealed that HP is an independent risk factor for PFS (HR= 1.90, 95%CI: 1.10-3.30; $P = 0.022$). Magahis *et al*[31] and other studies also found that the mPFS ($P < 0.01$) and OS ($P = 0.02$) of the HP-positive group were significantly shorter than those of the HP-negative group, and the OS of the positive group continued to be shorter after excluding patients receiving co-occurring chemotherapy (6.2 months *vs* 16.7 months).

The same multivariate analysis confirmed that HP is an independent determinant of PFS (HR = 3.04, $P < 0.01$) and OS (HR = 2.24, $P = 0.01$). Similar findings have been reported for other tumors, such as lung cancer[48] and melanoma[49]. There is no exact mechanism to explain this phenomenon, though it may be related to the tumor microenvironment. In fact, in addition to HP being the most common bacterium in the stomach, there are other common microbial communities in the stomach, and the *Proteobacteria phylum* is the second most common bacterial community in HP-positive GC[50]. Increasing evidence continues to suggest that other microbial communities in the stomach also promote GC development and the abundance and diversity of the gastrointestinal microbiome affects the efficacy of immunotherapy[51,52]. HP alters the gastrointestinal microbiome, and the abundance of some bacteria returns to normal after eradication of HP[53]. In fact, some studies have shown that the abundance of the gastrointestinal microbiome in HP-positive GC and HP-negative GC cases is different[54]. Therefore, we speculate that the long-term colonization of HP infection causes changes in the abundance of the gastrointestinal microbiome, leading to a negative correlation between immune treatment effects. Of course, the specific mechanism is not yet clear and further research is needed.

This meta-analysis also has some limitations. First, the number of studies available was limited, and they each included small cohort sizes. The relationship between HP infection and tumor PD-L1 expression in patients with GC needs to be verified by large sample studies, and it is best to have prospective double-blind controlled studies to support the data in the future. Second, there was significant heterogeneity in the included studies because this study is based on the meta-analysis of single-variate analysis studies. Other characteristics of HP infection and GC PD-L1 expression relationships were not analyzed, such as HP detection method, HP-related virulence factors (CagA, VagA), GC tissue type, microsatellite instability, and GC tumor-node-metastasis staging, among others. Finally, the conclusions of this study should be interpreted with caution as observational results. HP is a well-established GC pathogenic factor, and the importance of its eradication to prevent GC has been

recognized. Our study suggests that that HP increases PD-L1 expression in GC. Based on this result, whether patients with GC receiving immunotherapy should receive HP eradication treatment requires further verification.

CONCLUSION

In summary, the outcomes of the meta-analysis demonstrate that HP infection is related to tumor PD-L1 expression in GC patients. These results suggest that the status of HP infection may be beneficial in influencing the therapeutic efficacy of PD-1/PD-L1 blockade therapy in these patients, indicating that HP can be a potential indicator of GC immunotherapy prognosis. Nonetheless, the relationship between HP and immunotherapy requires further confirmation in the future.

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SIMILARITY INDEX

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