



Chemopreventive Effect of Metformin on Gastric Cancer Development

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Although *Helicobacter pylori* infection is the most important causative factor for gastric cancer (GC), *H. pylori* eradication alone does not completely eliminate the GC risk. In addition to *H. pylori* eradication, other risk factors for GC should be identified and targeted. Diabetes mellitus (DM) confers a 20% increased risk of GC, which could be mediated via several biological mechanisms including the stimulation of cell proliferation via hyperinsulinemia and increased insulin-growth factor production, the promotion of angiogenesis, and DNA damage. With a current global prevalence of 9.3% and a predicted rise to 10.2% by 2030, DM could contribute substantially to the burden of GC cases worldwide. Emerging evidence showed that metformin possesses chemopreventive effects via both direct (e.g., adenosine monophosphate-activated protein kinase activation and subsequent inhibition of the mammalian target of rapamycin pathway) and indirect (e.g., modulation of the interaction between tumor cells and their microenvironment and gut microbiota) pathways. A recent meta-analysis of observational studies showed that metformin use was associated with 24% lower GC risk. However, many available observational studies related to metformin effects suffered from biases including the failure to adjust for the *H. pylori* infection status and serial glycemic control and time-related biases. Future prospective studies addressing these pitfalls are needed. (**Gut Liver 2022;16:147-156**)

Key Words: *Helicobacter pylori*; Chemoprevention; Diabetes mellitus; Metformin; Gut microbiota

INTRODUCTION

Gastric cancer (GC) is the fifth most common cancer worldwide, with more than one million new cases (5.7% of all incident cancer cases) in 2018.¹ The incidence has great regional variability, being the highest in East Asia (in particular China, Japan, and Korea) where around half of the new cases are diagnosed, followed by Latin America and Eastern Europe. The overall 5-year survival rate is only 25% to 30%² as around two-thirds of patients are diagnosed at an advanced stage in which curative surgery is not possible.^{3,4} Despite the advances in chemotherapy, the prognosis remains dismal in patients with advanced disease, with a median survival of less than 1 year.

The most important causative factor for GC development is *Helicobacter pylori* infection with a relative risk of 2.8 as shown in a recent meta-analysis.⁵ *H. pylori* has been

classified as class I human carcinogen by the International Agency for Research on Cancer of the World Health Organization.⁶ With a global prevalence of *H. pylori* ranging from 19% to 88% in adults,⁷ it is estimated that *H. pylori* infection attributes to 89% of non-cardia GC cases, which in turn accounts for 78% of all GC cases.⁸ It is postulated that *H. pylori* infection triggers and promotes the Correa's gastric carcinogenesis cascade⁹—a sequential change of the gastric mucosa from chronic gastritis to atrophic gastritis, intestinal metaplasia, dysplasia, and finally adenocarcinoma. In a population-based cohort study, the risk of GC was increased in patients with atrophic gastritis, intestinal metaplasia and dysplasia as compared to those with normal gastric mucosa by a hazard ratio (HR) of 4.5, 6.2 and 10.9, respectively.¹⁰ Recently, intestinal metaplasia has been recognized as a surrogate biomarker of the genetic instability that promotes the progression of gastric stem cells to



cancer stem cells.

Although *H. pylori* is the most important cause of GC, eradication of *H. pylori* alone is not enough to eliminate the risk of subsequent GC development. A recent meta-analysis of seven randomized controlled trials (RCTs) of 8,323 subjects (six of which were conducted in East Asian countries), showed that *H. pylori* eradication reduced GC risk by only 46%.¹¹ The fact that a significant proportion of *H. pylori*-eradicated subjects progress to GC is likely related to the presence of baseline pre-cancerous lesions undermining the efficacy of *H. pylori* eradication in reducing GC risk.^{12,13} While *H. pylori* eradication reverses chronic gastritis and atrophic gastritis, prior studies showed that it may not regress intestinal metaplasia lesions,¹⁴⁻¹⁶ the presence of which was once considered a “point of no return” in the GC cascade.¹² Moreover, a recent prospective study with a follow-up duration of up to 10 years found no significant difference in the prevalence of intestinal metaplasia between *H. pylori*-eradicated and -infected subjects in their antrum and corpus, after the follow-up periods of ≥ 5 and ≥ 3 years, respectively.¹⁷ In contrast, a study that observed 2,258 patients for up to 15 years showed that *H. pylori* eradication reduced GC risk even in those with intestinal metaplasia and dysplasia.¹⁸ The above-mentioned meta-analysis also included three RCTs of 1,841 patients with confirmed gastric neoplasia who had undergone endoscopic resection, and showed that *H. pylori* eradication could still reduce the subsequent GC risk by about 51%.¹¹ Taken together, current evidence suggests that although eradication of *H. pylori* can reduce the GC risk and may regress precancerous gastric mucosal changes, the risk of GC remains in a considerable proportion of subjects even after *H. pylori* eradication.¹¹

DIABETES MELLITUS AS RISK FACTOR FOR GC

Apart from *H. pylori* infection, lifestyle factors could also potentially modulate the risk of precancerous lesions and GC including smoking, alcohol consumption, high salt intake, ingestion of nitrogen-containing compounds, vitamins and antioxidants.¹⁹ Family history of GC, genetic polymorphism and hereditary cancer syndrome are also contributing factors. Importantly, diabetes mellitus (DM) is a recognized risk factor of GC.²⁰

With a global prevalence of 9.3% in 2019 and a projected rise to 10.2% by 2030,²¹ the potential burden of GC attributable to DM could be substantial. Multiple biological mechanisms associating DM with GC have been proposed, which include the stimulation of cell proliferation

via hyperinsulinemia and increased insulin-growth factor (IGF) production,²² the promotion of angiogenesis by increasing vascular endothelial growth factor level,²³ DNA damage by the direct effect of hyperglycemia²⁴ and an indirect effect through an increased production of reactive oxygen species.²⁵ The carcinogenic effect of DM may also be partly mediated via excessive body weight (overweight and obesity), which is a commonly associated condition. A meta-analysis showed that excess body weight (body mass index ≥ 25 kg/m²) was associated with a 1.2-fold increase in GC risk, with stratified analysis showing an increased risk for cardia GC (odds ratio [OR], 1.55) but not non-cardia GC.²⁶ One possible mechanism is the increased risk of gastroesophageal reflux disease (GERD) among subjects with excess body weight, which is a known risk factor for adenocarcinomas of the gastroesophageal junction.²⁷ Other proposed mechanisms of how excess body weight increases GC risk include insulin resistance with elevated levels of IGFs, alterations in the levels of adiponectin, leptin, sex steroids and glucocorticoids, obesity-related inflammatory markers, oxidative stresses, and nuclear factor- κ B (NF- κ B) system, resulting in perturbation in the normal balance between cell proliferation, differentiation, and apoptosis.^{26,28}

A population-based cohort study of 2,603 Japanese subjects showed the GC risk was higher with higher hemoglobin A1c (HbA1c) levels.²⁹ The age- and sex-adjusted incidence of GC among individuals with HbA1c levels of 5.0% to 5.9%, 6.0% to 6.9%, and $\geq 7.0\%$ were 2.5, 5.1, and 5.5 per 1,000 person-years. *H. pylori* infection and a higher HbA1c level ($\geq 6.0\%$) had a synergistic effect on GC risk.

There are six meta-analyses investigating the effect of DM on GC risk in the past decade (Table 1). A meta-analysis of 17 observational studies in 2013 showed that DM is associated with a 19% higher GC risk.²⁰ However, there are few studies reporting the effect of DM on GC according to cancer subsite. Li *et al.*¹⁸ found that there was a significant association between self-reported DM and cardia cancer risk (HR, 1.89; 95% confidence interval [CI], 1.43 to 2.50), while Kim *et al.*³⁰ refuted this association (HR, 0.64; 95% CI, 0.14 to 2.94). However, the individual results included in the meta-analysis showed conflicting results. A subsequent meta-analysis of 15 cohort studies in 2017 refuted the association (pooled risk ratio [pooled RR], 1.10; 95% CI, 0.94 to 1.29).³¹ This inconsistency could be due to a failure to adjust for *H. pylori* infection status and concomitant medication usage (including proton pump inhibitors [PPIs], aspirin, non-aspirin nonsteroidal anti-inflammatory drugs [NA-NSAIDs], cyclooxygenase [COX]-2 inhibitors, statins and metformin), which may modulate the risk of GC (to be explained in more detail in next section). The latter potentially biases a positive association between DM

Table 1. Summary of Meta-Analyses on the Association between Diabetes Mellitus and Gastric Cancer

Author (year)	Literature search	Study design	No. of included studies	Pooled risk ratio (95% CI)
Sona <i>et al.</i> (2018) ³⁹	Inception till April 2017	Cohort studies (type I diabetes mellitus)	9	1.44 (1.29–1.61)
Miao <i>et al.</i> (2017) ³¹	Details not available	Cohort studies	15	1.10 (0.94–1.29)
		Eastern countries	5	0.97 (0.72–1.32)
		Western countries	10	0.92 (0.75–1.14)
Yoon <i>et al.</i> (2013) ²⁰	Inception till February 7, 2012	5 Case control studies + 11 cohort studies + 1 nested case control study	17	1.19 (1.08–1.31)
		Eastern countries	7	1.19 (1.02–1.38)
		Western countries	10	1.18 (1.03–1.36)
Shimoyama (2013) ³⁸	1950 to January 2013	2 Case control studies + 9 cohort studies	11	1.41 (1.10–1.81)
		Eastern countries	5	1.77 (1.38–2.26)
		Western countries	6	1.23 (0.90–1.68)
Tian <i>et al.</i> (2012) ⁴⁰	Inception till October 10, 2011	7 Case control studies + 18 cohort studies	25	1.11 (1.00–1.24)
Ge <i>et al.</i> (2011) ⁴¹	Inception till May 31, 2011	4 Case control studies + 17 cohort studies	21	1.09 (0.98–1.22)
		Asia	6	1.30 (0.95–1.80)
		Europe	8	1.00 (0.79–1.26)
		North America	7	0.96 (0.90–1.02)

CI, confidence interval.

and GC to null, as a higher proportion of patients with DM may require treatment with aspirin, statins, and metformin, which could potentially harbor chemopreventive effects against GC development. One study also found that the association between DM and subsequent GC risk varied over time, with increasing risk of GC being observed only more than 4 years after diagnosis of DM.³²

Our recent territory-wide cohort study of 46,460 subjects showed that type II DM was associated with an increased GC risk (adjusted HR [aHR], 1.73; 95% CI, 1.08 to 2.79) even after *H. pylori* eradication, particularly in those with HbA1c $\geq 6.0\%$. This association was only found in cardia GC (HR, 3.4; 95% CI, 1.45 to 7.97), but not in non-cardia GC (HR, 1.53; 95% CI, 0.84 to 2.78).³³ This differential effect of DM on cancer subsite may be attributed to the association between obesity and GERD and hence cardia cancer. Eradicating *H. pylori* restores gastric acid production by improving corpus inflammation, which may worsen GERD.³⁴

It should also be noted that among those meta-analyses which showed a significant association between GC and DM, subgroup analysis showed the harmful effect of DM was limited to Asians but not non-Asians (Table 1). While this geographical difference may be due to higher prevalence of GC risk among Asian countries, the intricate interplay between *H. pylori* infection (with a higher prevalence in the Asians) and metabolic syndrome including DM on GC development may also play a role.^{35–37} Another major limitation of current studies is the failure to distinguish between type I and type II DM, which differ significantly in terms of their metabolic characteristics.³⁸ Nevertheless, as type I DM is less frequent than type II DM, majority of patients included in the meta-analyses can be reasonably

regarded as having type II DM. Remarkably, there is one meta-analysis including nine studies of type I DM patients only showing a pooled RR of 1.44.³⁹ This harmful effect appears to be more prominent compared with other meta-analyses without differentiation of the types of DM. Further studies are warranted to investigate a potential differential effect of the two types of DM on GC development.

PHARMACOLOGICAL AGENTS IN THE MODIFICATION OF GC RISK

Increasing evidence has emerged to show that certain medications may modulate GC risk. However, majority of these studies included a heterogeneous population of *H. pylori*-infected and *H. pylori*-negative subjects. PPIs lead to profound acid suppression, which could worsen atrophic gastritis,⁴² particularly in *H. pylori*-infected subjects.^{43,44} The increase in gastrin (a potent growth factor that have trophic effect on the gastric mucosa) in response to this resultant hypochlorhydria stimulates enterochromaffin-like cell hyperplasia.⁴³ A meta-analysis of seven studies showed that PPIs were associated with a 2.5-fold higher GC risk.⁴⁵ This risk was noted even after *H. pylori* eradication.⁴⁶ However, although an association has been shown, the causality between PPIs and GC development warrants further investigation as current evidence is still conflicting. In a nested case-control study with 1,233 GC cases,⁴⁷ PPI use of ≥ 2 years was not associated with higher risk of GC and consistent association was not found for increasing PPI dose. However, in exploratory analyses, PPI use of ≥ 10 years was associated with a 30% higher GC risk. In a 3 \times 2 partial factorial double-blinded RCT involving

17,598 subjects with cardiovascular disease and peripheral artery disease (and hence were given combination of aspirin and rivaroxaban, aspirin alone or rivaroxaban), participants were randomly assigned to receive pantoprazole 40 mg daily or placebo.⁴⁸ There was no statistically significant difference in multiple safety events except for an increased enteric infection among pantoprazole users. However, this study only reported all gastrointestinal cancers (n=169) without specifying the number of GC cases. It will not be surprising that this study was indeed much underpowered to study the outcome of GC as evidenced by the few cases of gastric atrophy (n=45). Moreover, the median duration of follow-up was only 3 years, which was relatively short to study GC outcomes. Another issue is that a significant proportion of the study participants had concomitant use of aspirin, which is a potential chemopreventive agent against GC.⁴⁹ In fact, *post hoc* analysis of the territory-wide cohort study showed that PPI-associated GC risk could be offset by concomitant use of aspirin.⁵⁰

Aspirin and statins are relatively inexpensive medications for the treatment and prevention of cardiovascular events. They are also found to possess potential chemopre-

ventive effects against solid organ tumors.⁴⁹ Statins exert this effect via halting cell-cycle progression,⁵¹ inducing apoptosis,⁵² inhibiting angiogenesis,⁵³ and inhibiting the growth of tumor cells.⁵⁴ Aspirin inhibits COX-2 and non-COX pathways,^{55,56} such as phosphatidylinositol 3-kinase, NF- κ B,⁵⁷ Wnt- β -catenin, extracellular signal-regulated kinase, and activated protein 1.⁵⁸ Meta-analyses of clinical studies reported that the uses of aspirin⁴⁹ and statins⁵⁹ were associated with 36% and 32% lower risks of GC respectively. The beneficial effects of both agents are also observed after *H. pylori* eradication.^{60,61}

COX-2 overexpression is detected in gastric intestinal metaplasia and cancer.⁶² Two RCTs have been conducted to investigate the effect of COX-2 inhibitors on intestinal metaplasia.^{63,64} One study showed that rofecoxib use for 2 years did not regress intestinal metaplasia or its severity in *H. pylori*-eradicated subjects with intestinal metaplasia.⁶³ Another study showed that celecoxib use for 2 years regressed advanced gastric lesions in *H. pylori*-infected but not *H. pylori*-eradicated subjects.⁶⁴ As for NA-NSAIDs, our recent territory-wide cohort study of 92,017 *H. pylori*-eradicated subjects found that NA-NSAID use was not

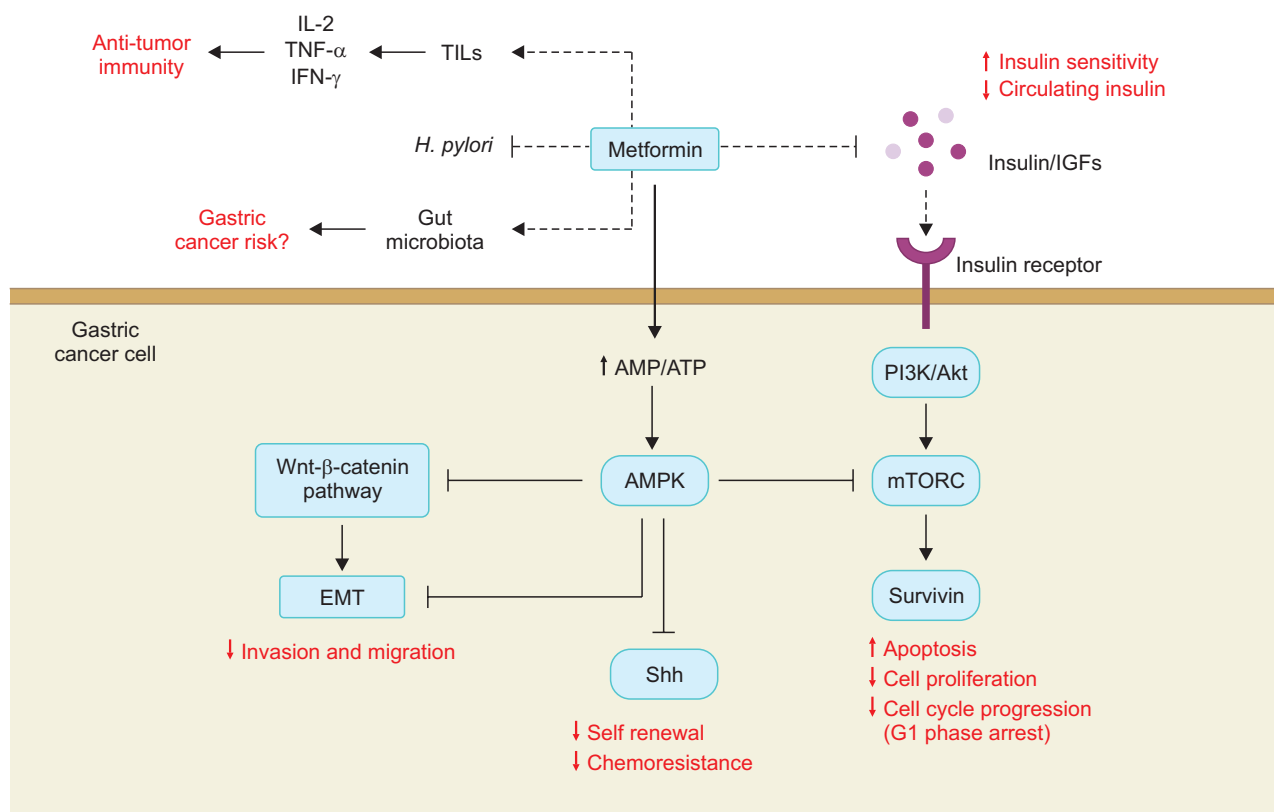


Fig. 1. Mechanisms underlying the chemopreventive effects of metformin.

IL-2, interleukin-2; TNF- α , tumor necrosis factor α ; IFN- γ , interferon γ ; TILs, tumor-infiltrating lymphocytes; *H. pylori*, *Helicobacter pylori*; IGFs, insulin-growth factors; AMP, adenosine monophosphate; ATP, adenosine triphosphate; AMPK, AMP-activated protein kinase; EMT, epithelial-to-mesenchymal transition; Shh, sonic hedgehog; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; mTORC, mammalian target of rapamycin complex. Solid lines and dotted lines represent direct and indirect effects of metformin on gastric cancer development.

associated with a lower risk for GC.⁶⁵ An updated meta-analysis in this study showed that the pooled adjusted RR was 0.88 (95% CI, 0.77 to 1.01).

METFORMIN IN REDUCING GC DEVELOPMENT

1. Anti-tumorigenic mechanisms of metformin on GC development

The anti-cancer activity by metformin is proposed to be mediated by several pathways (Fig. 1). First, as metformin is an insulin sensitizer, it reduces the production of insulin and IGFs. This cripples the IGF signaling pathway that facilitates the proliferation of cancer cells expressing IGF receptors.²² Secondly, the activation of adenosine monophosphate-activated protein kinase (AMPK) and the subsequent inhibition of the mammalian target of rapamycin complex 1 pathway and survivin (one of the inhibitor of apoptosis proteins family that is associated with poor prognosis in GC)^{66,67} is shown to induce cancer cell apoptosis.^{68,69} Other potential direct anti-carcinogenic mechanisms of metformin include anti-proliferative effect on gastric cell lines causing G1 cell-cycle arrest,^{70,71} the inhibition of the Wnt- β -catenin pathway,⁷² a reversal of epithelial-to-mesenchymal transition,⁷³⁻⁷⁵ AMPK-dependent inhibition of the sonic hedgehog signaling pathway,⁷⁶ which is essential for the maintenance of self-renewal and chemoresistance of cancer stem cells, and targeting cancer stem cells and progenitor cells.⁷⁷ It is noteworthy that these anti-tumorigenic effects of metformin are also applicable to other solid organ cancers.⁷⁸ A number of meta-analyses of cohort and case-control studies have revealed a 10% to 40% lower overall cancer risk.⁷⁸ In a meta-analysis of 41

observational studies of more than one million patients with type 2 DM, metformin was associated with a lower cancer risk of liver (OR, 0.34; 95% CI, 0.19 to 0.60), colorectum (OR, 0.83; 95% CI, 0.74 to 0.92), pancreas (OR, 0.56; 95% CI, 0.36 to 0.86) and esophagus (OR, 0.90; 95% CI, 0.83 to 0.98).⁷⁹ Other meta-analyses have also reported lower cancer risk of breast, prostate, endometrium and lung with metformin use.⁷⁸

Indirect effects of metformin include the modulation of (1) the interaction between GC cells and their micro-environment and (2) gut microbiota.⁸⁰ Co-culturing GC cells with metformin-pretreated gastric tumor-associated fibroblasts was observed to reduced GC cell proliferation.⁸¹ In addition, *in-vivo* and *in-vitro* experiments have demonstrated antibiotic properties of metformin in inhibiting *H. pylori*, in a dose-dependent manner.⁸² Metformin also induced solid tumor rejection in mice via an activation of CD8 + tumor-infiltrating lymphocytes (TILs). With uninhibited cancer growth, TILs progressively lose their ability to secrete interleukin-2, tumor necrosis factor α , and interferon γ and eventually undergo apoptosis in an immune exhaustion process. Metformin treatment prevented this TILs-apoptosis and induced TILs migration into tumors to reactivate anti-tumor immunity.⁸³ It has recently been shown that metformin is able to modulate gut microbiota,⁸⁴ Whether the gut microbiota changes induced by metformin will in turn influence GC risk necessitates further investigations.

2. Chemopreventive effect of metformin on GC development in clinical studies

Table 2 summarizes the results of five recent meta-analyses on the potential chemopreventive effect of metformin on GC. While protective effects with varying effect

Table 2. Summary of Meta-Analyses on the Potential Chemopreventive Effect of Metformin on Gastric Cancer

Author [year]	Literature search	Study design	No. of included studies	Pooled risk ratio (95% CI)
Wang <i>et al.</i> (2021) ⁸⁵	Inception till August 9, 2020	Cohort studies	14	0.78 (0.69–0.88)
		Presence of immortal time bias	6	0.67 (0.59–0.77)
		Absence of immortal time bias	8	0.95 (0.85–1.05)
Shuai <i>et al.</i> (2020) ⁸⁶	Inception till October 22, 2019	Cohort studies	11*	0.79 (0.62–1.00)
		Asian population	3	0.54 (0.38–0.78)
		Western population	5	0.99 (0.99–0.99)
Zhou <i>et al.</i> (2017) ⁸⁷	Inception till November 2016	Cohort studies	7	0.76 (0.64–0.91)
		China	4	0.51 (0.38–0.69)
		Outside of China	3	0.90 (0.80–1.02)
Li <i>et al.</i> (2017) ⁸⁸	Inception till June 30, 2016	Cohort studies	5	0.87 (0.73–1.04)
Franciosi <i>et al.</i> (2013) ⁷⁹	January 1966 till April 2012	Cohort studies	2	0.83 (0.76–0.90)
		Randomized controlled trials (<i>post-hoc</i> analysis)	2	0.48 (0.11–2.02)

CI, confidence interval.

*There are 6 studies in West and 5 studies in Asia. However, for subgroup analysis, the authors only report the results for 3 Asian studies and 5 Western studies.

estimate were shown for some studies,^{89,90} others failed to demonstrate such association.⁹¹⁻⁹⁵ A meta-analysis of seven cohort studies in 2017 showed that metformin was associated with a 24% lower GC risk,⁸⁷ but a recent meta-analysis of 14 cohort studies reported a similar magnitude of effect (21% lower risk) albeit with borderline significance (95% CI, 0.63 to 1.00; $p=0.051$). Subgroup analysis showed that the beneficial effect was limited to Asians (pooled RR, 0.54; 95% CI, 0.38 to 0.78) but not the Western population. Due to a lack of RCTs, the chemopreventive role of metformin in GC remains controversial based on data from observational studies. As an example, in two population-based retrospective cohort studies, the baseline characteristics differed between metformin users and non-users, including age, sex, socioeconomic status, concomitant use of other oral anti-diabetic medications and glycemic control.^{92,96} Hence, the seemingly beneficial effect of metformin on GC prevention may be due to the difference in these risk factors instead. Despite the use of multivariable analysis or propensity score analysis, residual confounding may still be present.⁹⁷

Nonetheless, a dose- and duration-response relationship may help to strengthen the possibility of causality. In a territory-wide cohort study in Hong Kong, the aHRs of GC with a cumulative defined daily dose (cDDD) of <975 (median cDDD) and ≥ 975 was 0.73 (95% CI, 0.35 to 1.53) and 0.33 (95% CI, 0.13 to 0.86), respectively, when compared with metformin non-users.⁹⁶ This study also showed that the GC risk was lower with increasing duration of metformin use (aHR, 0.85; 95% CI, 0.74 to 0.96 for every 1 year increase in use); this effect size was similarly reported in a Korean nationwide cohort study (aHR, 0.88; 95% CI, 0.81 to 0.96).⁹² Both studies reported the beneficial effect was observed only among those who used metformin for at least 3 years (aHR, 0.35; 95% CI, 0.16 to 0.80; aHR, 0.57; 95% CI, 0.37 to 0.87, respectively).^{92,96}

Importantly, other important risk factors of GC such as *H. pylori* infection, and DM severity have not been adequately addressed in previous studies, potentially undermining the effect of metformin on GC prevention. As *H. pylori* is the most important risk factor of GC, failure to stratify patients according to *H. pylori* status will affect the true effect estimate of metformin on GC development. Moreover, GC risk was shown to be higher among individuals with higher HbA1c levels²⁹ and fasting plasma glucose.⁹⁸ *H. pylori* infection and a higher HbA1c level ($\geq 6.0\%$) had a synergistic effect on GC risk.²⁹ The protective effect of metformin on GC may therefore be due to a better glycemic control instead of the anti-cancer effects. This study, however, did not adjust for the effect of various medications and comorbidities, and therefore the independent

role of HbA1c level remains to be investigated. In addition, the severity of DM affects the use of other DM drugs, which may confound the observed chemopreventive effect of metformin on GC. It has been reported that metformin significantly reduced GC risk in non-insulin users only,⁹² as insulin use may increase cancer risk via the insulin and IGF signaling systems.

To address the confounding factors of *H. pylori* infection, glycemic control and insulin use, we have previously conducted a territory-wide study of 7,266 DM patients with *H. pylori*-eradicated and followed up for a median of 7.1 years.⁹⁶ The overall glycemic effect in addition to other covariates were considered in the multivariable analysis. As HbA1c varies with time, the time-weighted average HbA1c was used to represent the overall glycemic control during the observation period, which was derived as the average HbA1c weighted by the time interval between successive measurements. We found that metformin use was associated with a reduced GC risk (aHR, 0.49; 95% CI, 0.24 to 0.98). This association was independent of glycemic control. Our findings support the plausible biological mechanisms that metformin prevents GC not through better glycemic control. However, the limited number of GC outcomes ($n=37$) did not allow an in-depth study of whether this beneficial effect of metformin effect differs between the cardia and non-cardia GC.

Other concerns of the chemopreventive effect of metformin are time-related biases, including immortal time bias and time-window bias.⁹⁹ Immortal time bias arises when there is an inappropriate exclusion of immortal time or an erroneous assignment of immortal time to the exposed group due to the definition of exposure.¹⁰⁰ This immortal time biases the results towards an overestimation of the beneficial effects of a treatment. A recent meta-analysis of 14 cohort studies by Wang *et al.*⁸⁵ showed that pooled RR from six cohort studies with immortal time bias was 0.67 (95% CI, 0.59 to 0.77), while no beneficial effect of metformin (pooled RR, 0.95; 95% CI, 0.85 to 1.05) was shown from eight cohort studies without immortal time bias (Table 2). Meta-regression showed that presence of immortal time bias was associated with a reduction of 29% in the pooled RR. For cohort study design, this bias can be addressed by treating the exposure as a time-dependent covariate in the survival analysis modeling (in which the observation period is disintegrated into certain intervals and medication usage is defined in each interval) instead of time-fixed analysis. On the other hand, time-window bias arises in the context of case-control studies due to use of time-windows of different lengths between cases and controls to define time-dependent exposures.¹⁰¹ Nested case-control studies are less likely to suffer from immortal

time bias and time-window bias as a result of incidence-density sampling. However, the scientific value of positive studies that have not addressed immortal time bias should not be indiscriminately disregarded. This is evidenced by a similar conclusion on the beneficial effect of metformin between time-dependent analysis (by treating metformin as time-varying covariates)¹⁰² and time-fixed analysis in our territory-wide study of *H. pylori*-eradicated DM patients.⁹⁶

While RCTs may be difficult to be conducted due to the long lag time and low incidence of GC development, future nested case control or cohort studies should address the flaws of current studies including confounding effect of *H. pylori* infection status, glycemic control and time-related biases in addition to other known risk factors of GC.

CONCLUSIONS

GC remains a common cancer with poor prognosis. Although *H. pylori* infection is the most important causative factor of GC, DM is another risk factor that has a synergistic effect with *H. pylori* infection on increasing GC risk. Metformin has been shown to potentially modify the GC risk, even after *H. pylori* eradication. The chemopreventive effect of metformin appears to be independent of glycemic control. Nevertheless, current studies are limited to observational studies and future prospective studies addressing various concerns and biases are needed to fully discern the potential role of metformin in preventing GC.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Study conception: K.S.C., W.K.L. Acquisition of data: K.S.C. Drafting of manuscript: K.S.C., K.L.C. Revision of manuscript, and final approval of manuscript: W.K.L.

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